HIV, HBV, HCV Virology

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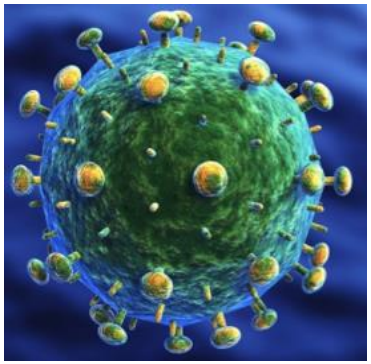
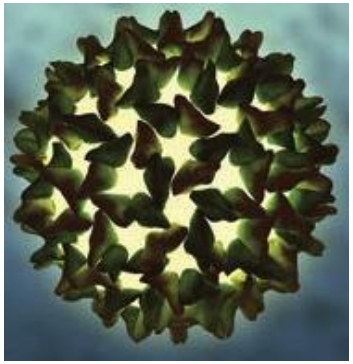
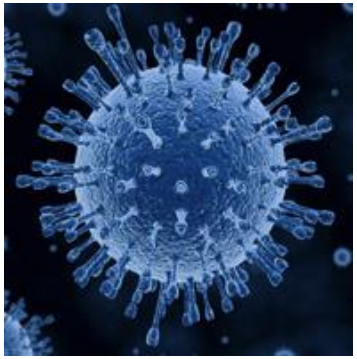
HIV



HBV



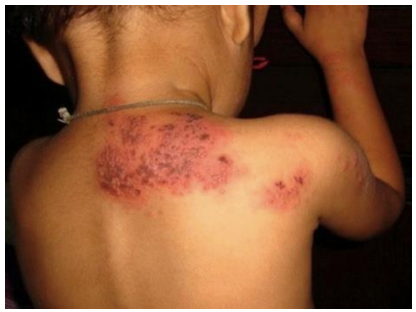
HCV

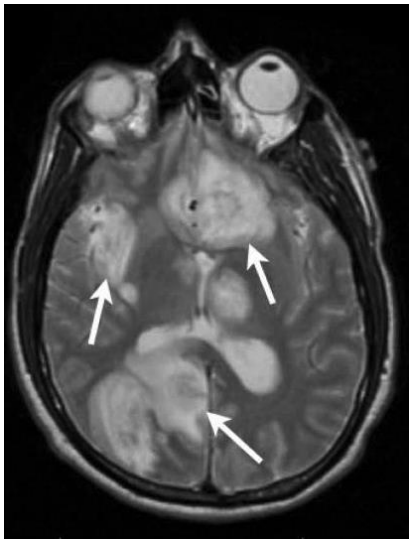


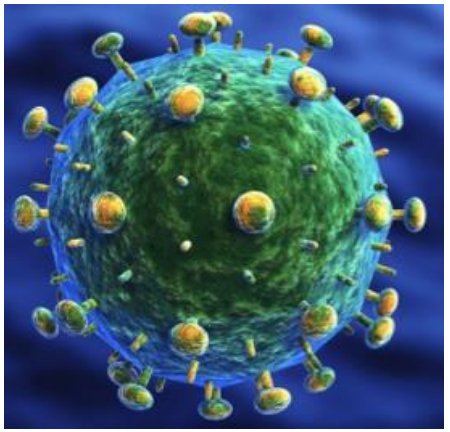
- **Many similarities**
- **Several fundamental differences**

HIV

RNA virus	• Chronic infection • Without treatment, most people develop AIDS and die within ~10 years (7.5 to 11.6)[1,2] • Non-AIDS HIV-related disease	• Latent reservoir as integrated provirus • Antiviral therapy controls but does not eradicate HIV • Life-long therapy required to suppress virus replication • PrEP and PEP



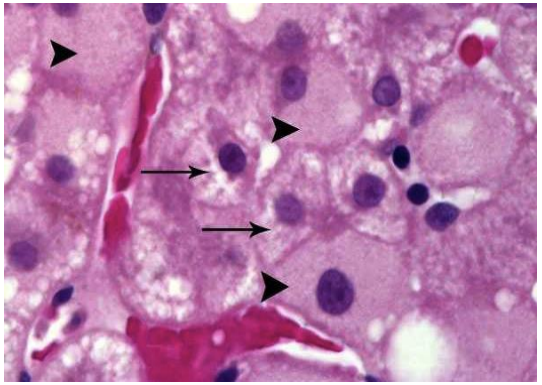


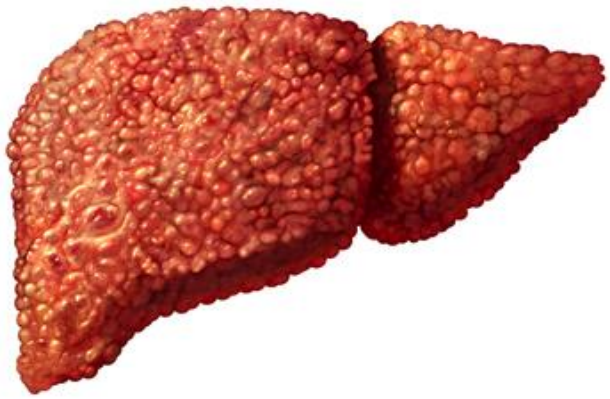


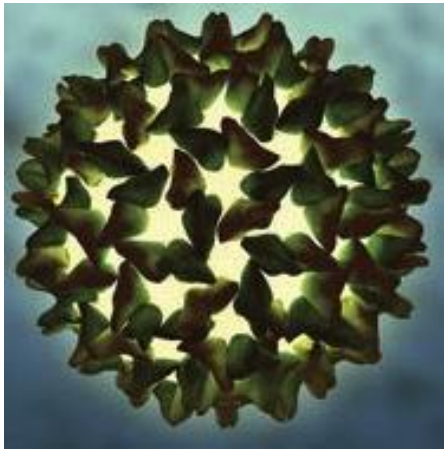
1. Todd AIDS 2007; 2. Laskey Nature Rev Microbiol 2014

HBV

DNA virus	• Vaccine • Chronic infection in >90% children, <5% adults • Cirrhosis (~30%) • Hepatocellular carcinoma (with/without cirrhosis) • Extra-hepatic disease	• Persistence as cccDNA, may integrate • Several replicative states • Antiviral therapy not always required, controls but does not eradicate HBV, can be stopped in some cases • Antivirals work as PrEP

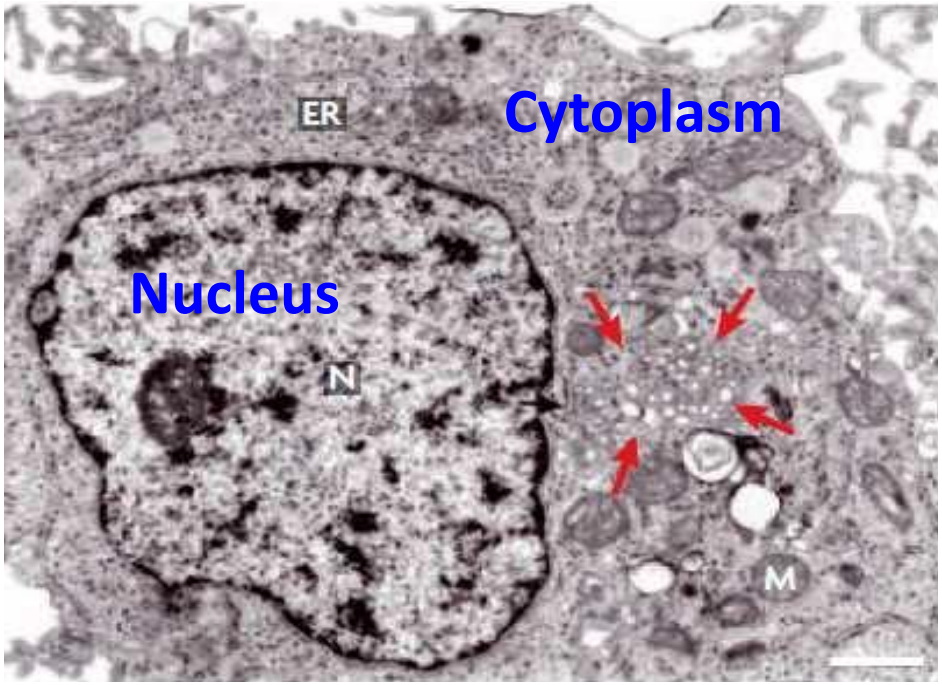


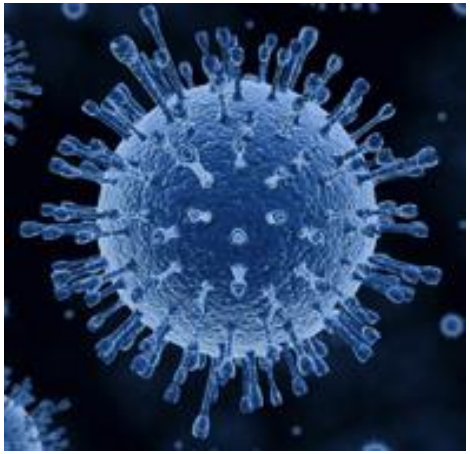




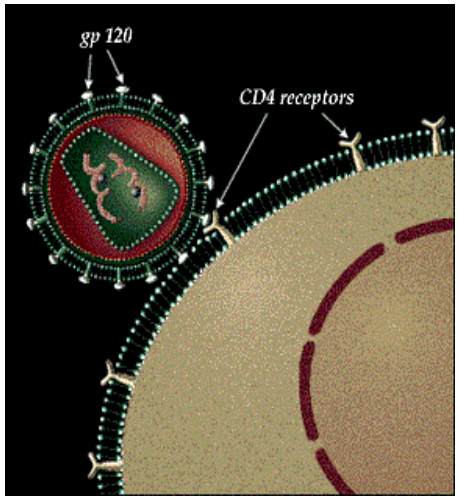
HCV

RNA virus	• Chronic infection ~80% • Cirrhosis (41% over 30 years), hepatocellular carcinoma • Extra-hepatic disease increasingly recognised[1,2]	• No stable or latent reservoir • Curable with antiviral therapy

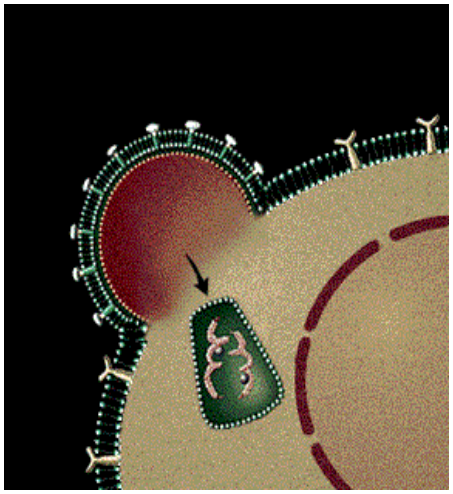




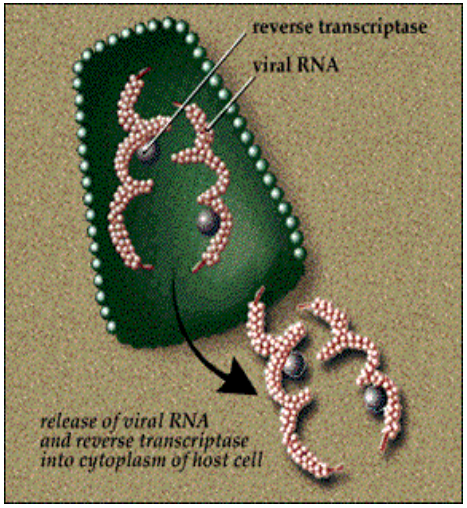
1. Giordano JAMA 2007; 2. Lee JID 2012



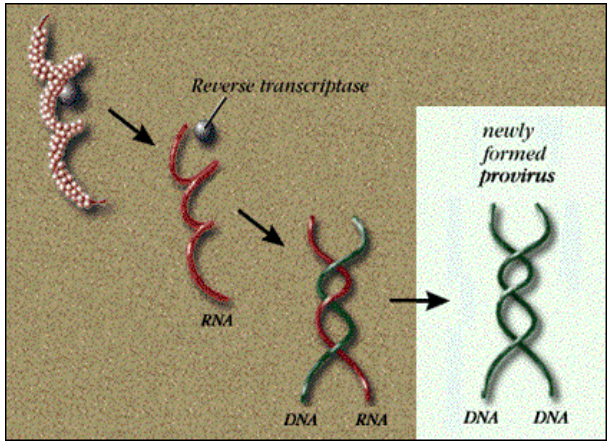
Attachment



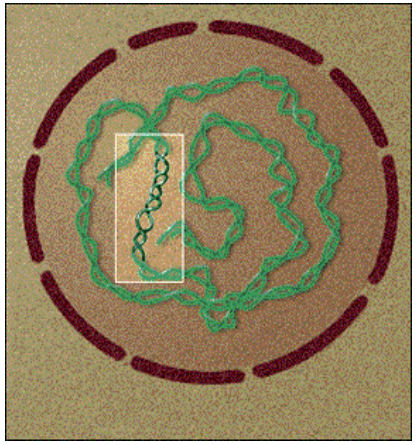
Fusion



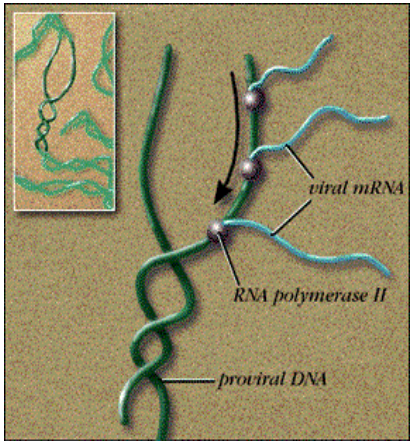
Release of RNA



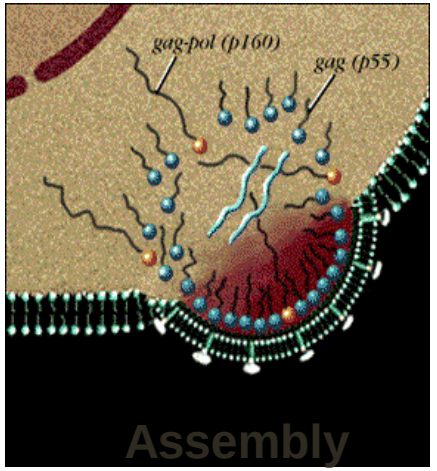
Reverse transcription

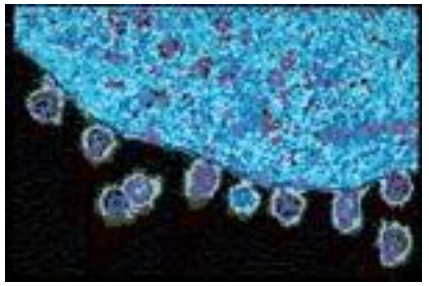


Integration



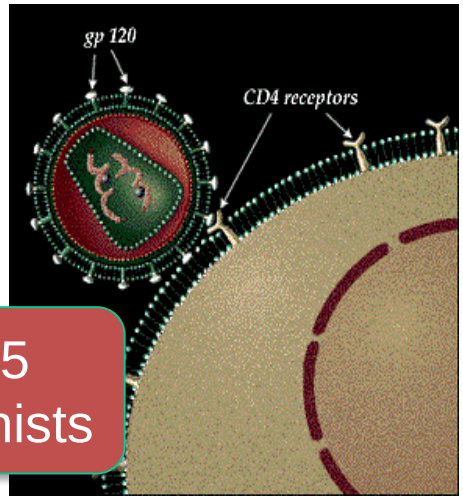
Transcription





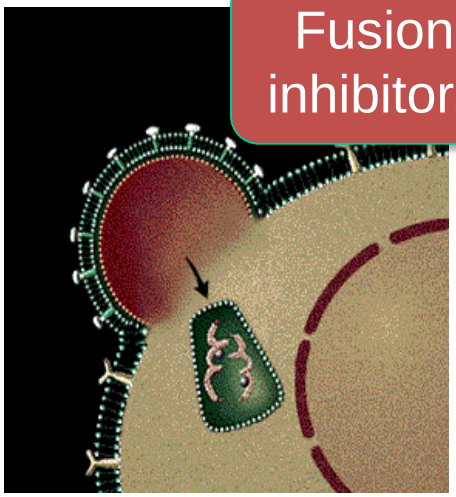
Maturation
& budding

HIV replication



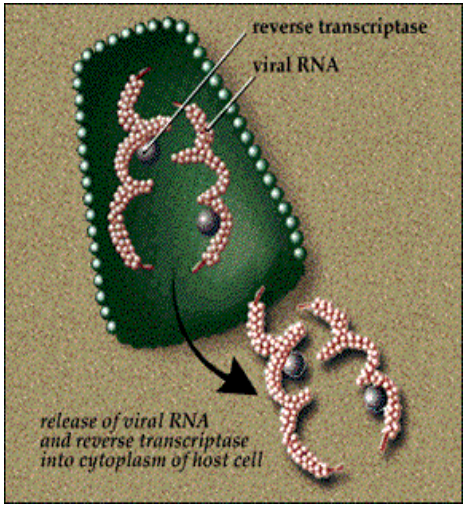
CCR5 antagonists

Attachment

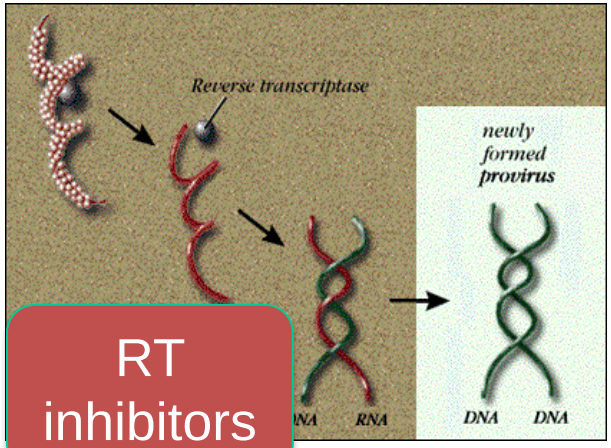


Fusion inhibitors

Fusion



Release of RNA

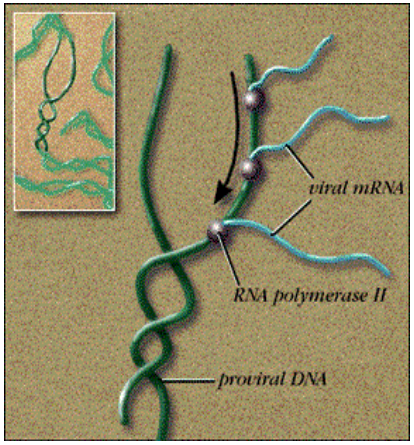


RT inhibitors

Reverse transcription

Integrase inhibitors

Integration



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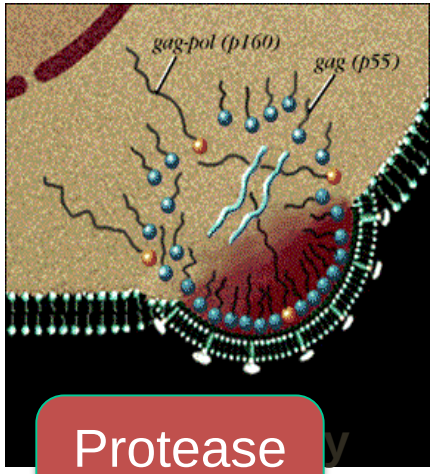
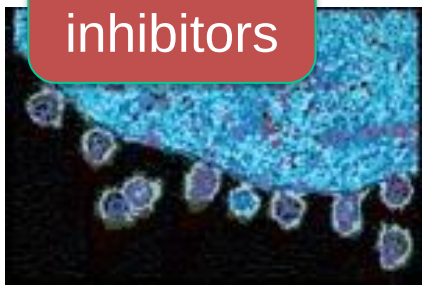


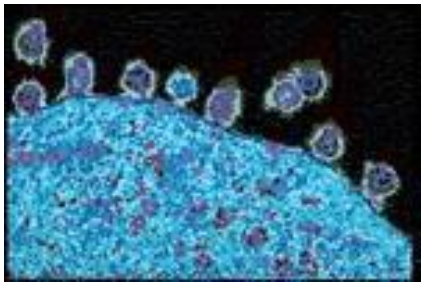
Protease inhibitors

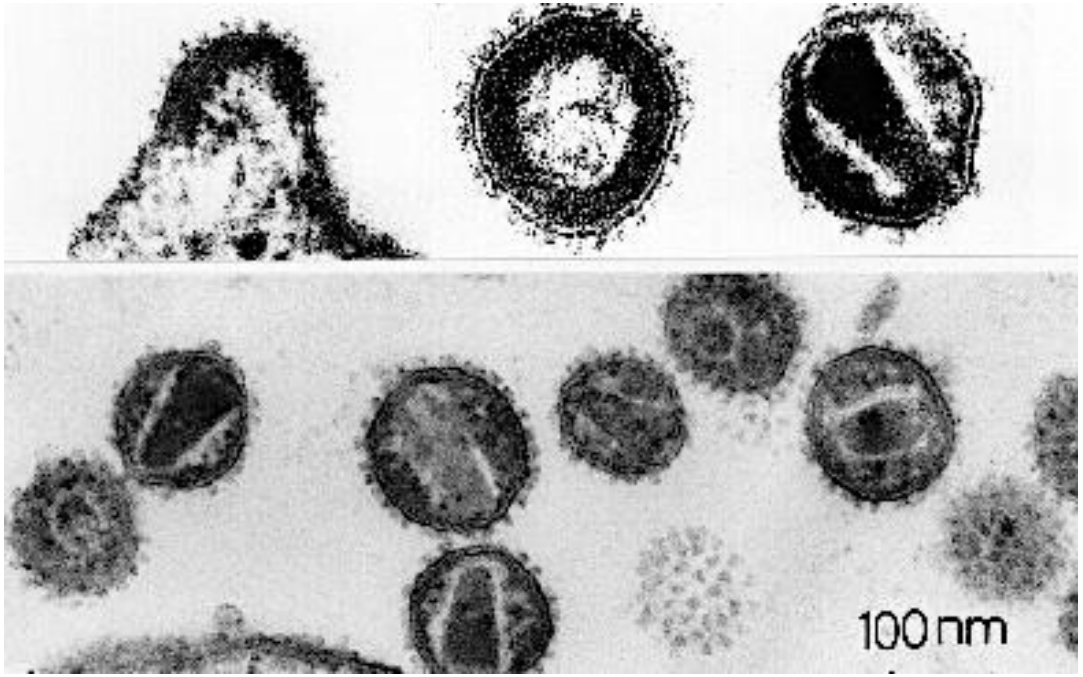


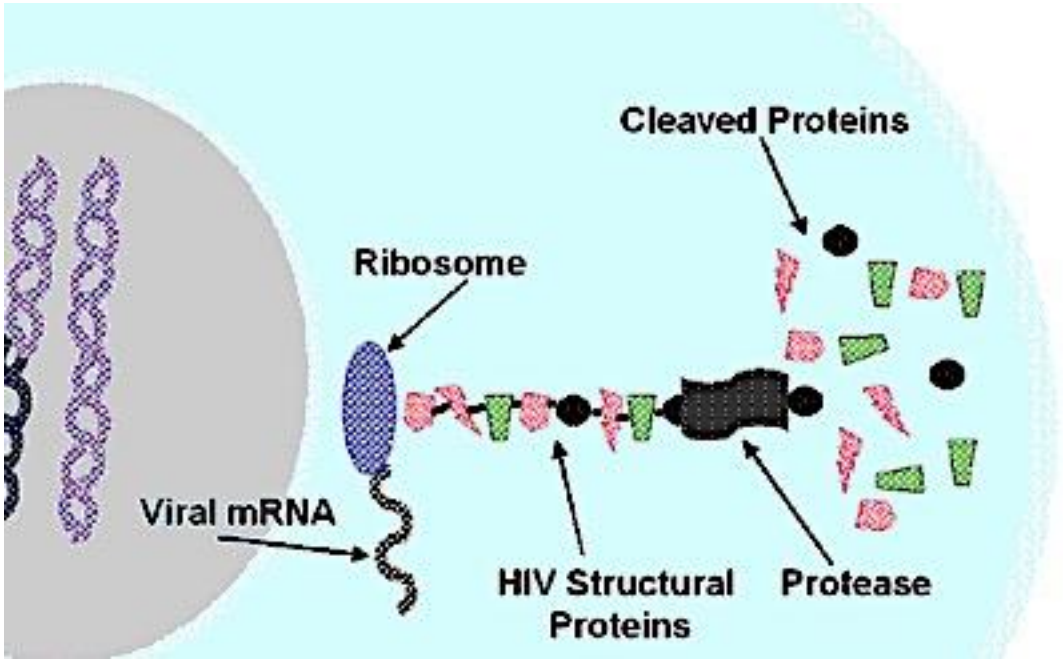
Maturation & budding

Targets of therapy

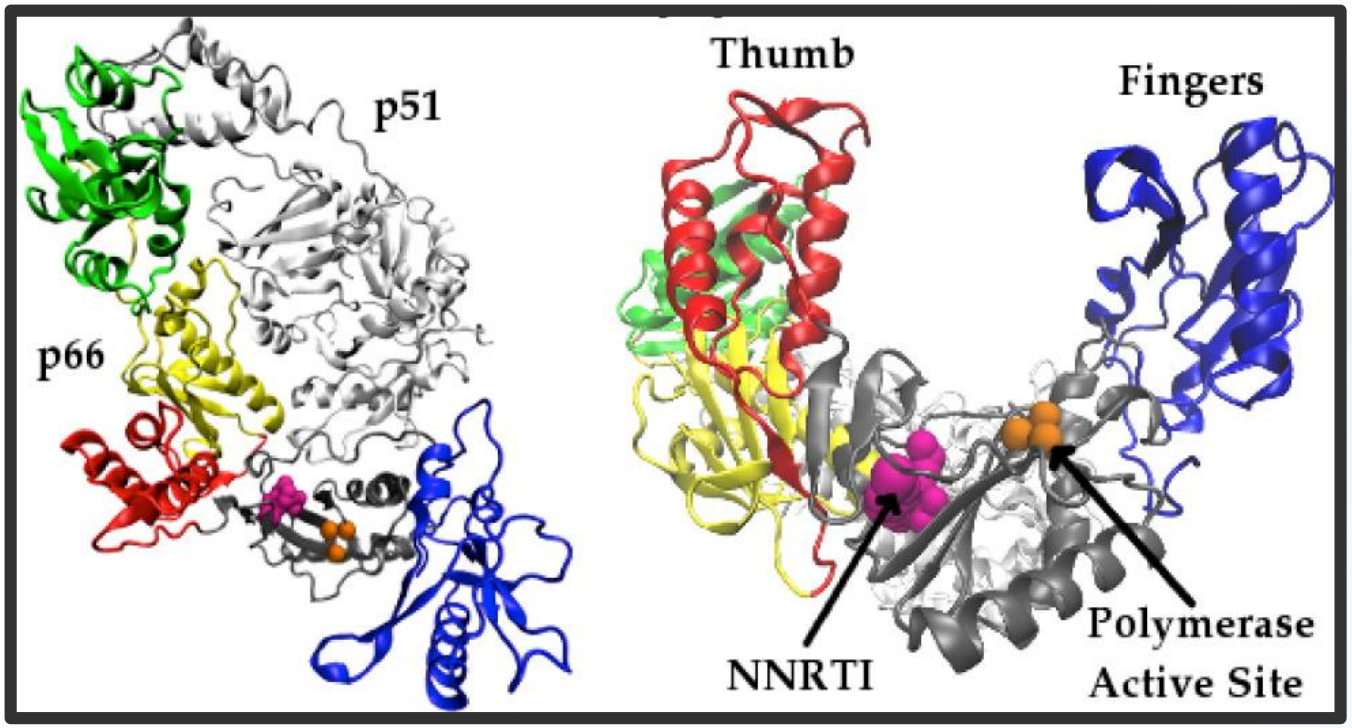
Maturation & budding







Polyprotein

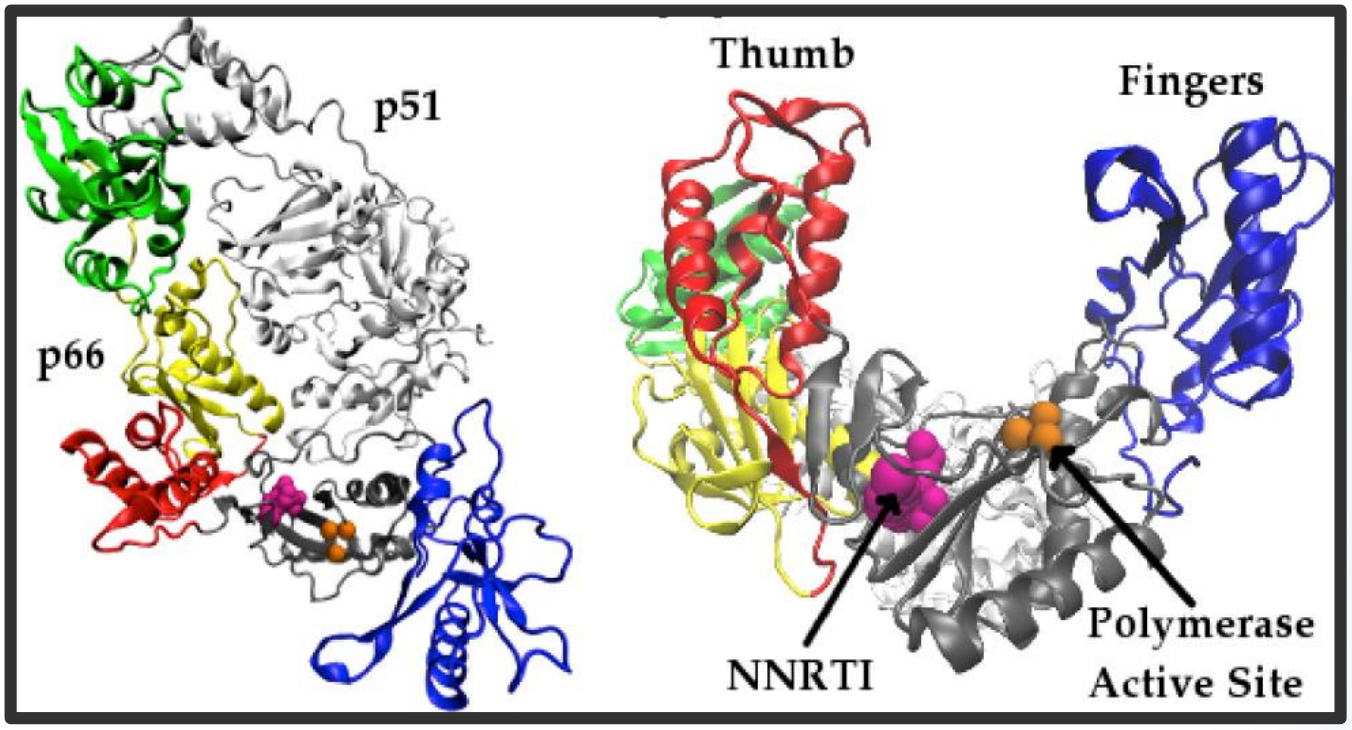


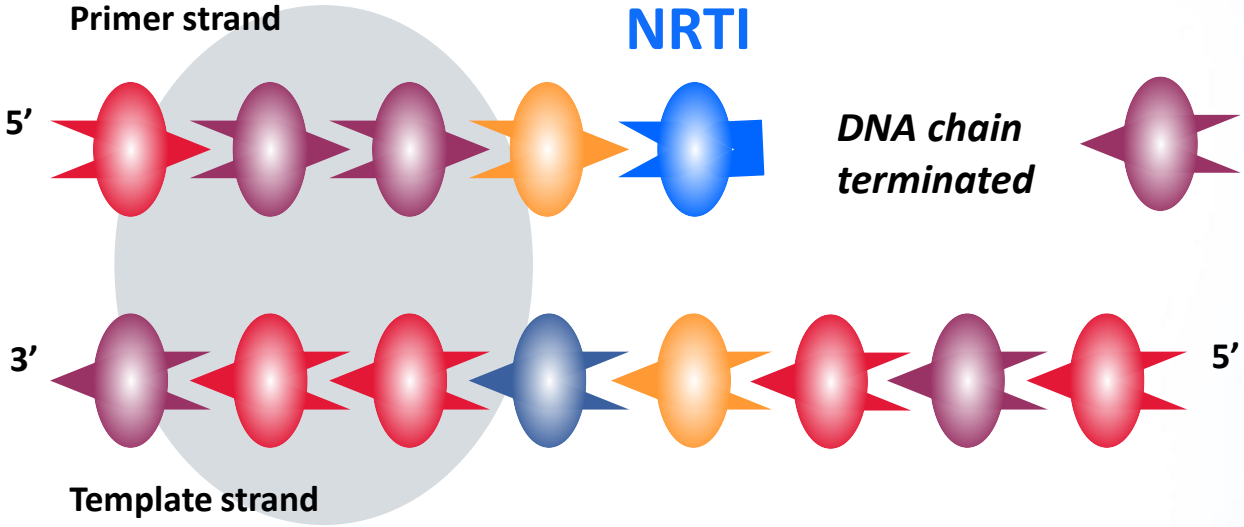
- **HIV Reverse transcriptase/Polymerase**

Two mechanisms of inhibition

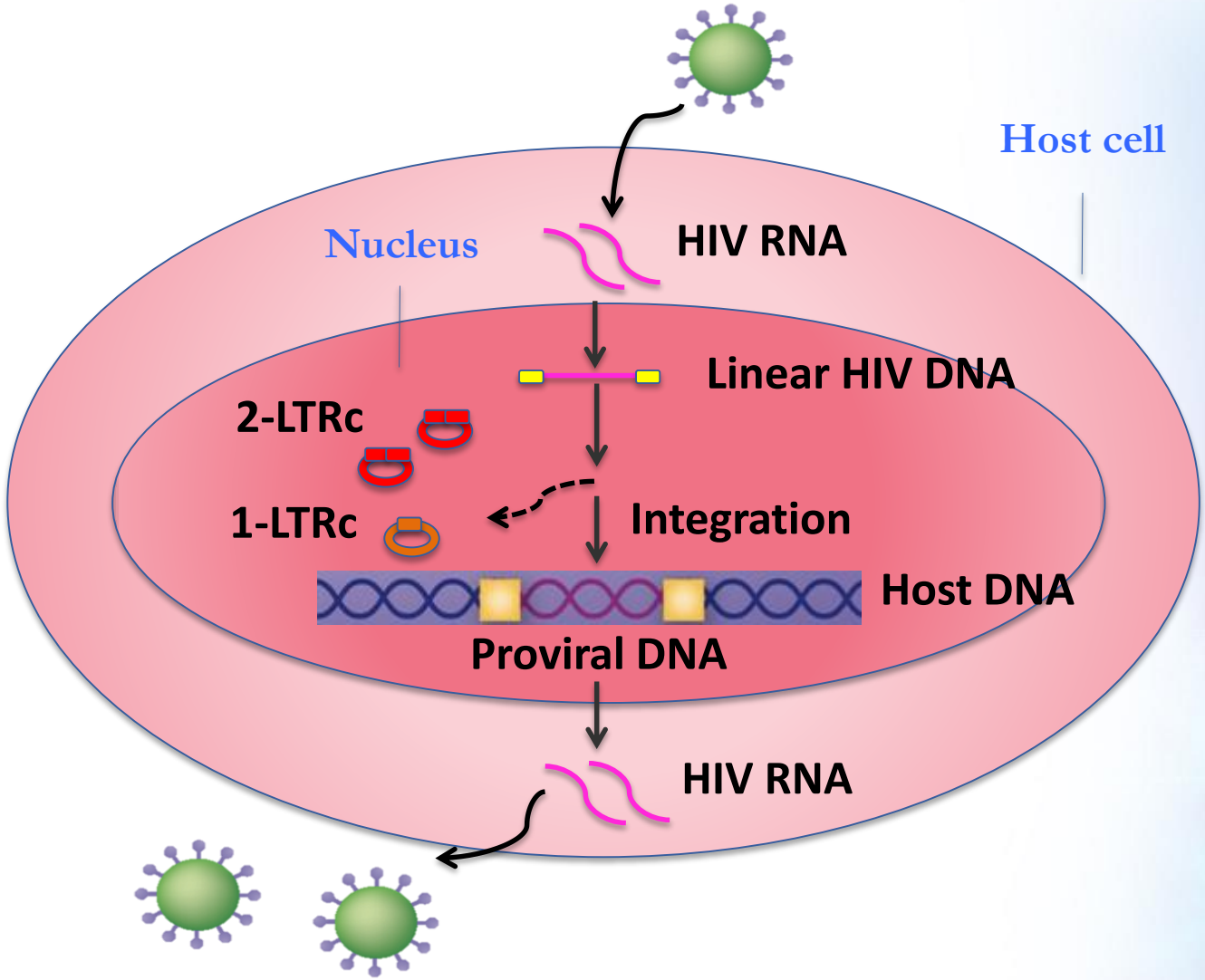
- **Competitive – NRTIs**
- **Allosteric – NNRTIs**

Wright et al. Biology 2012

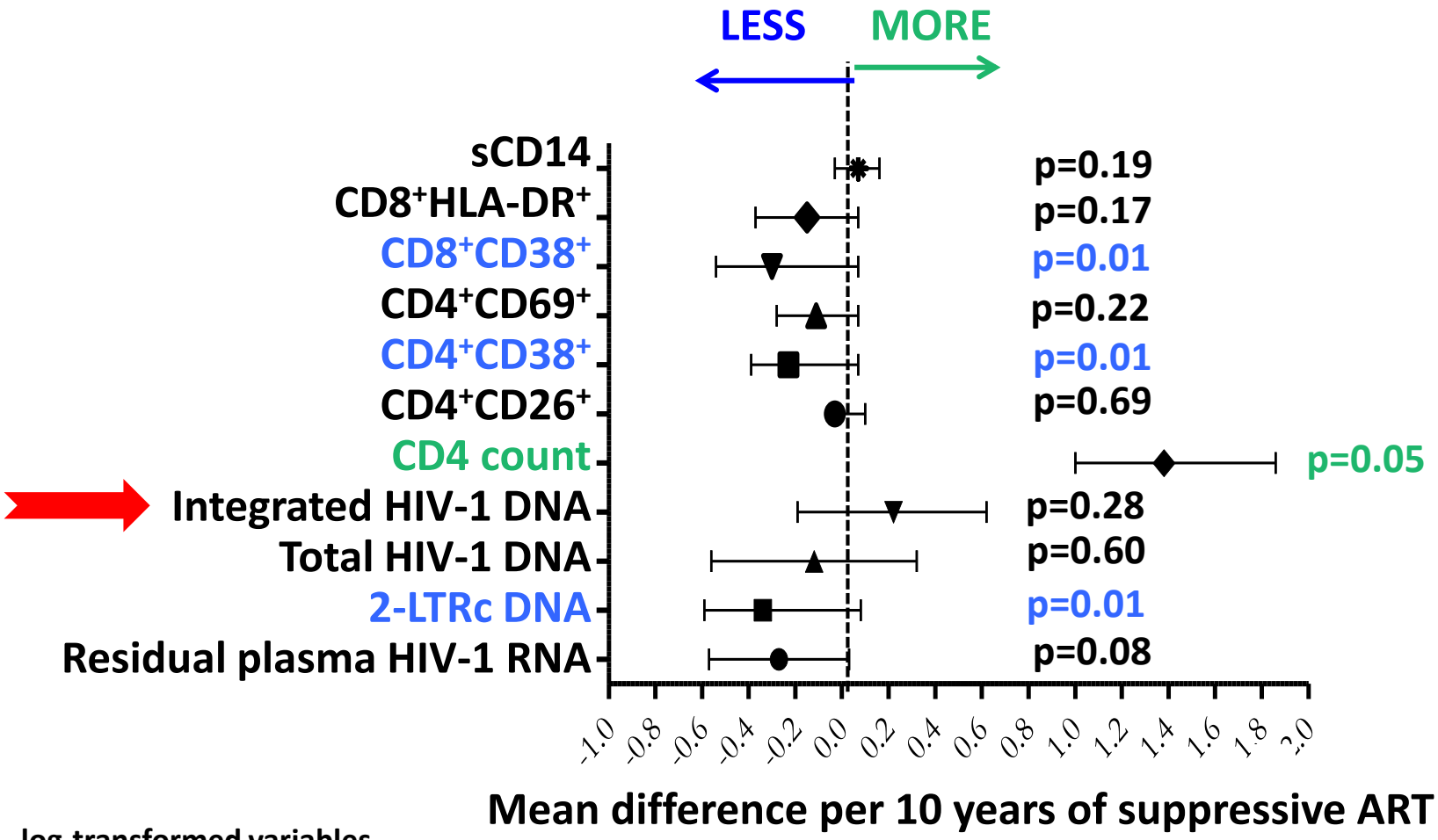

p51
p66
Thumb
Fingers
NNRTI
Polymerase
Active Site


Primer strand
NRTI
5’
DNA chain
terminated
3’
5’
Template strand

HIV DNA forms



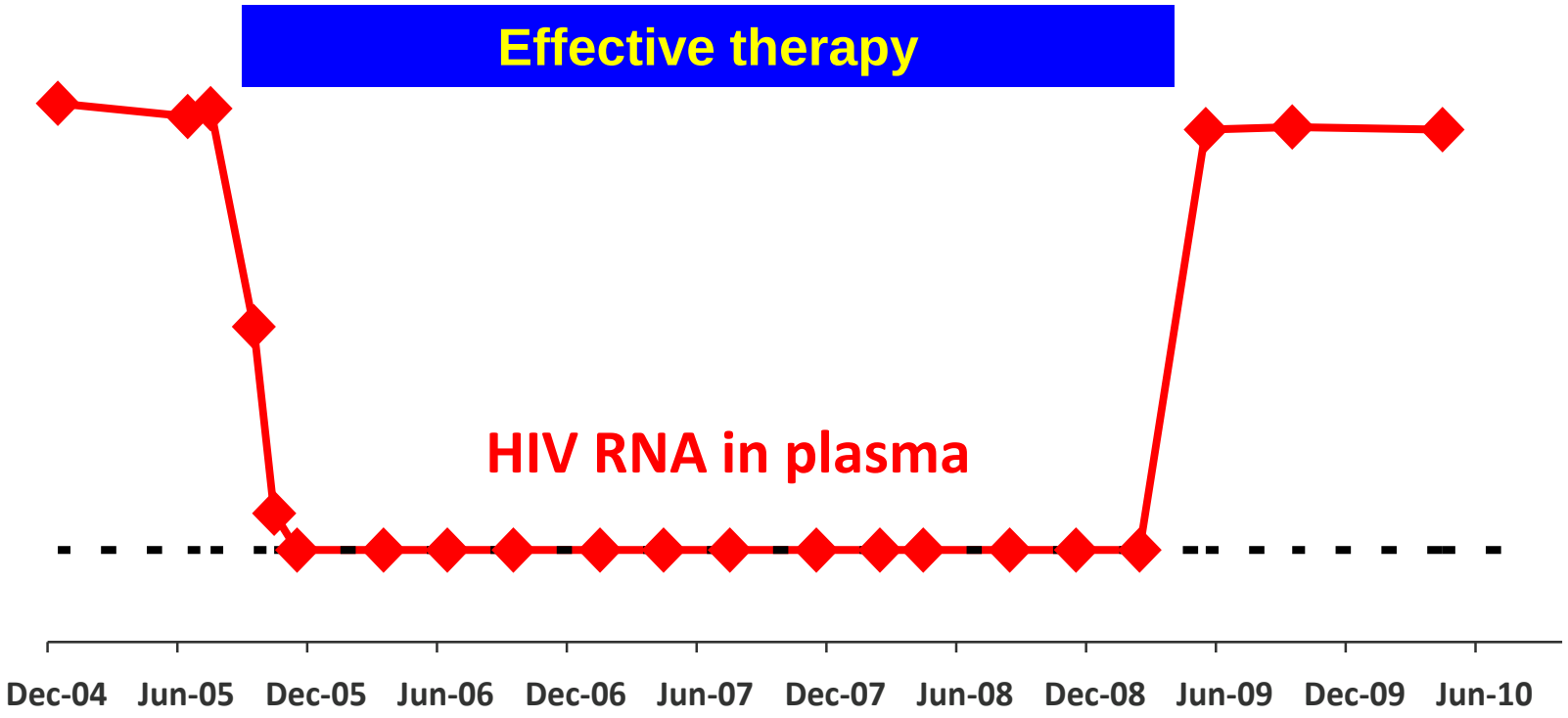
Effect of ART duration on virological & immunological parameters



log-transformed variables

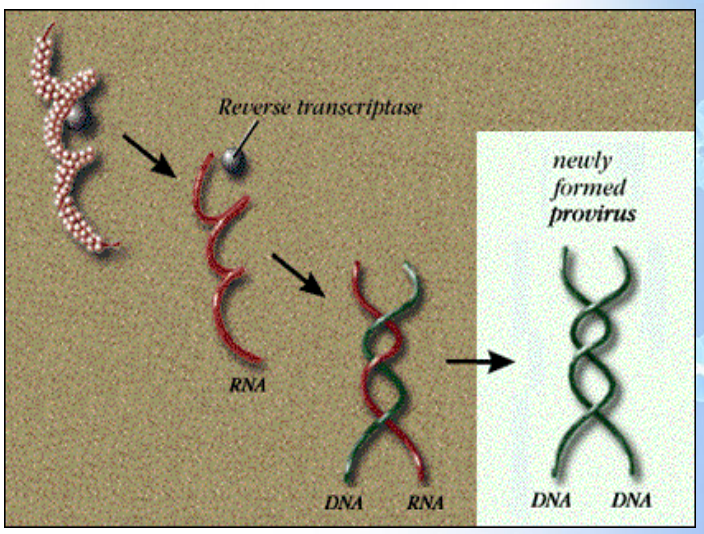
Ruggiero EBiomed 2015

Virus replication resumes if therapy is stopped



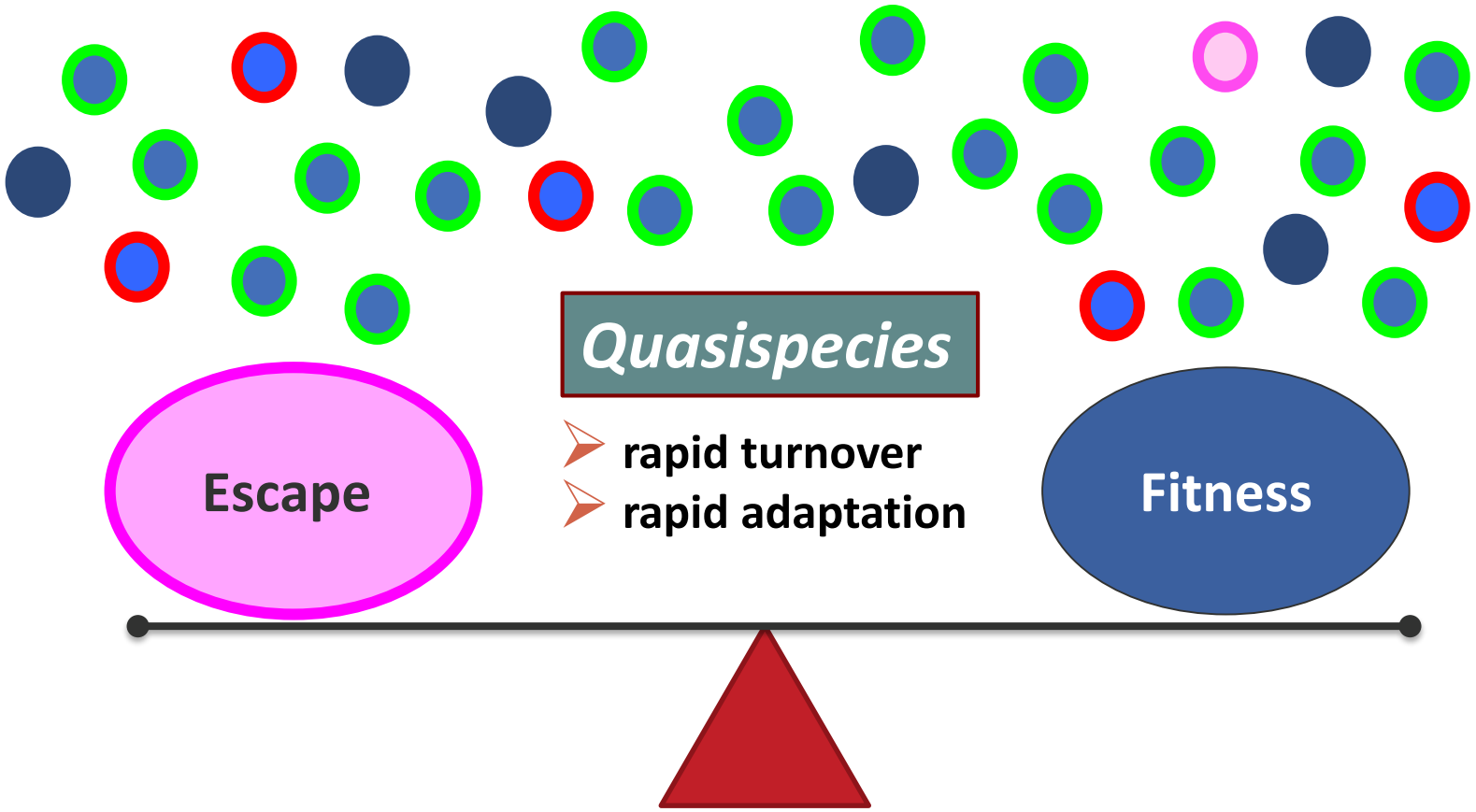
- Antiretroviral therapy cannot achieve HIV eradication
- After stopping therapy HIV replication resumes to pre-treatment levels

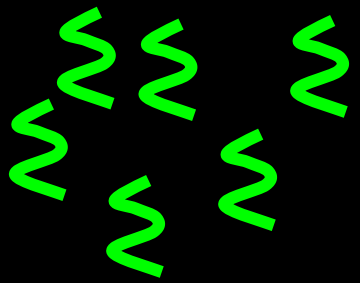
Mechanisms of HIV genetic evolution



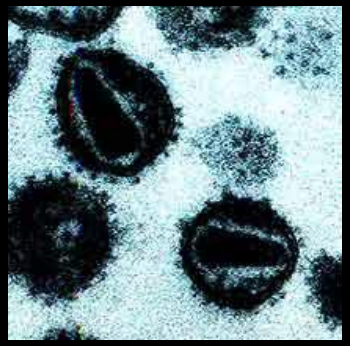
1. **Errors by viral reverse transcriptase**
   - ***~1 mis-incorporation per genome round***
2. Errors by cellular RNA polymerase II
3. APOBEC-driven G➔A hypermutation
   - *Deamination of cytosine residues in nascent DNA*
4. Recombination between HIV strains

- Rapid replicating virus (~10^{10} particles/day)
- Rapid clearance of newly produced virus
- Highly error prone polymerase ➔ High mutation rate
- Some mutations detrimental, some allow escape





PCR



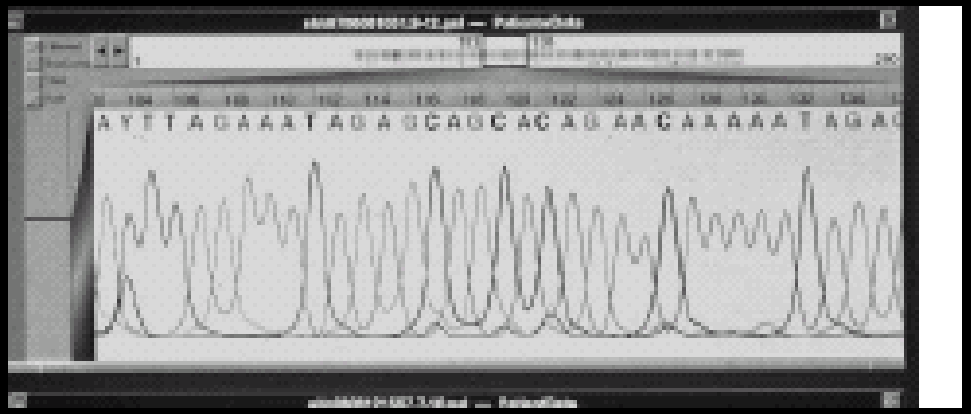
Viral gene (e.g., RT)

HIV RNA

Plasma

Sequencing

Mutations

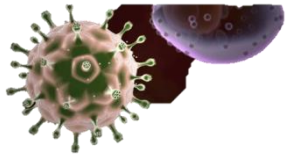


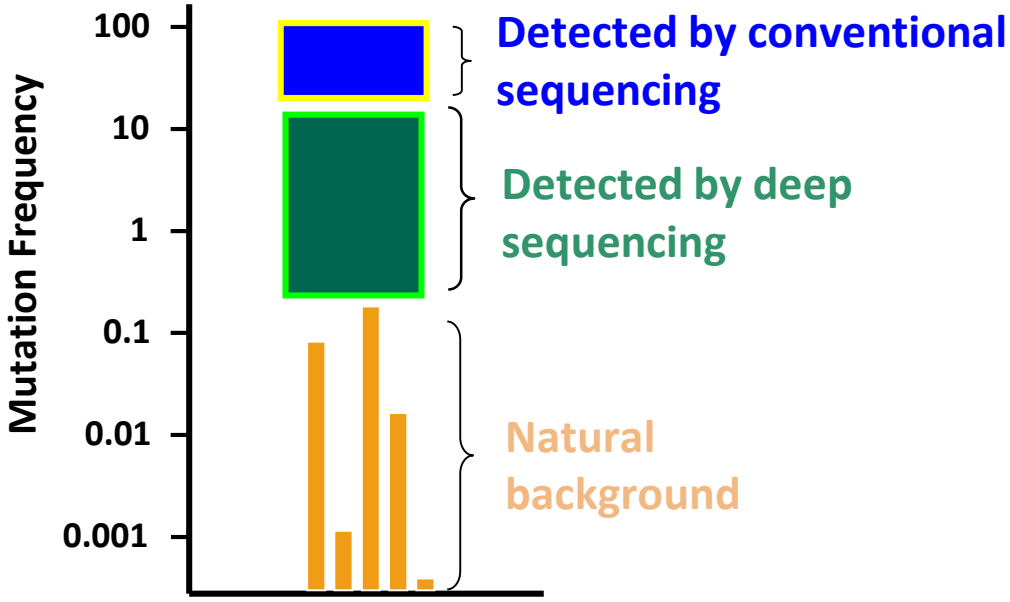
RT M184V

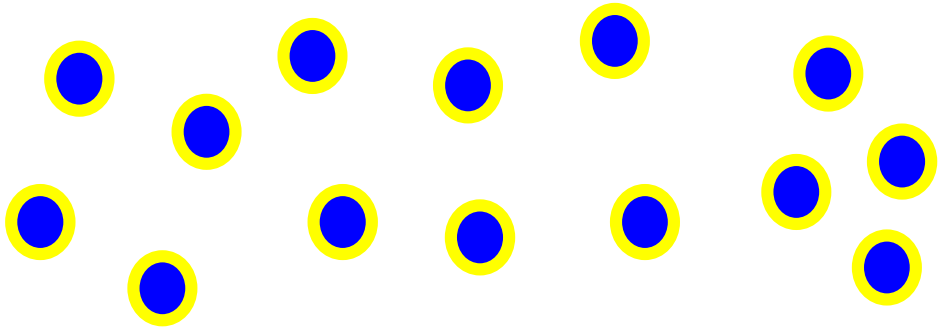
Methionine → Valine

@ codon 184 of RT

ATG / AUG → GTG / GUG

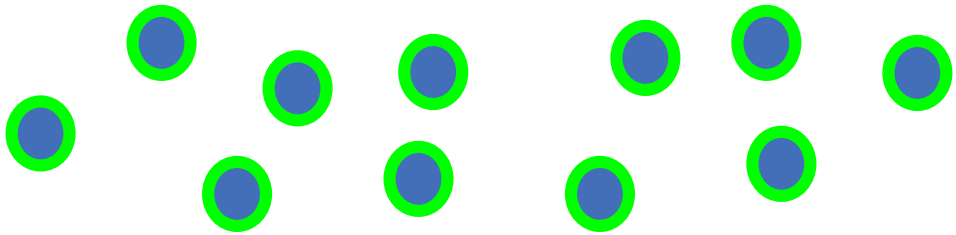




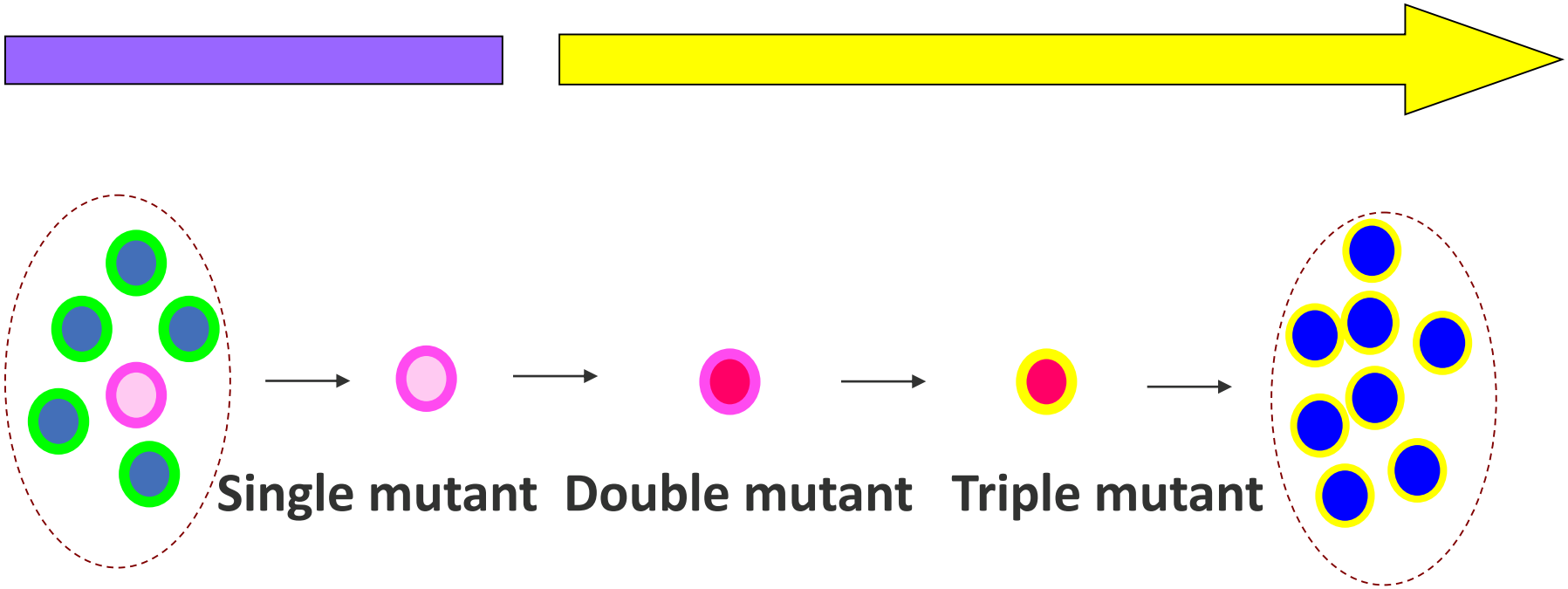


Limit of detection of conventional sequencing

~10-20%



Emergence & evolution of HIV drug resistance



The genetic barrier to resistance is expression of multiple interacting factors

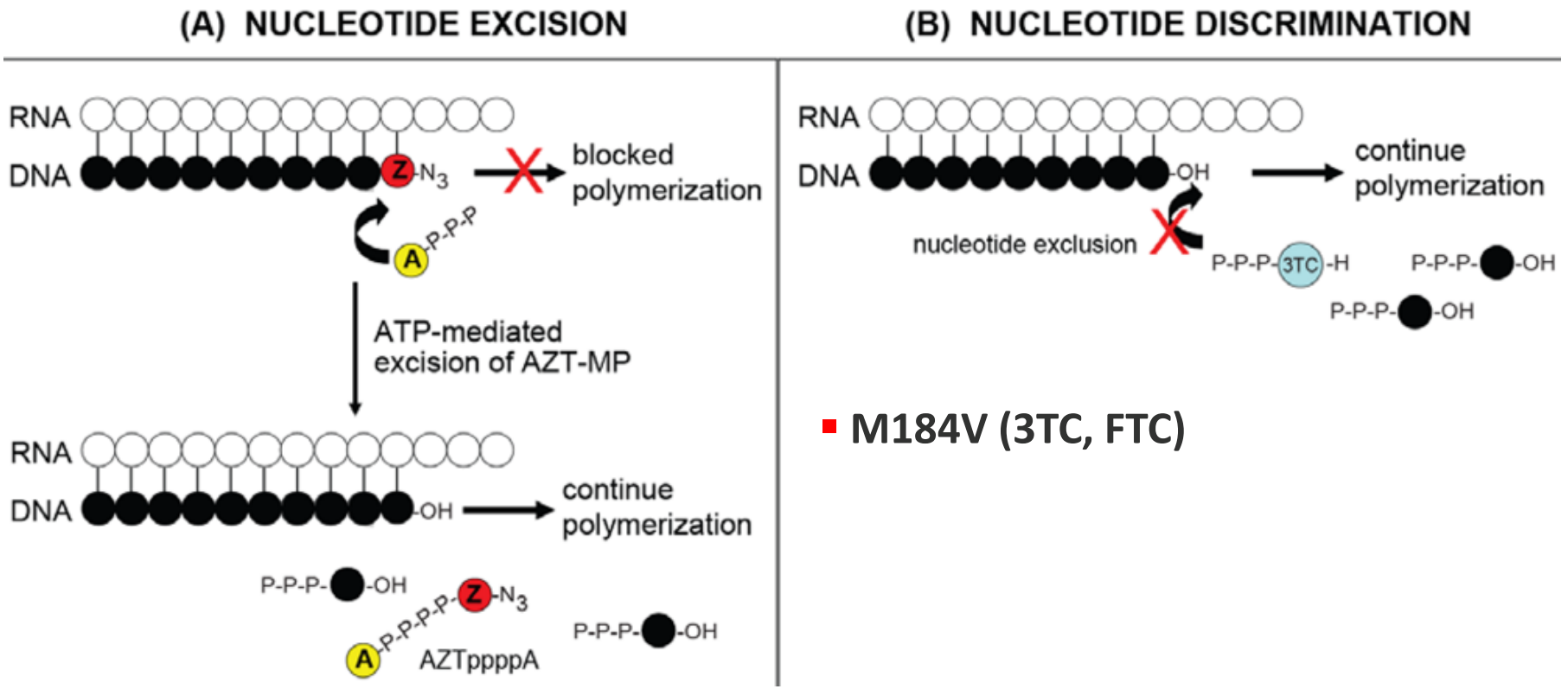
- Virus sequence
- Phenotypic effect of individual mutations
- No. of mutations required to reduce drug susceptibility
- Fitness cost of the mutation
- Ease of emergence of compensatory adjustments

- Drug potency
- Mode of interaction between drug and target
- Drug concentration
- Drug combination
- Antagonism or synergism between resistance pathways

- Viral load
- Host genetics
- Host immune function
- Reservoirs of replications

More than the sum of each drug in a regimen

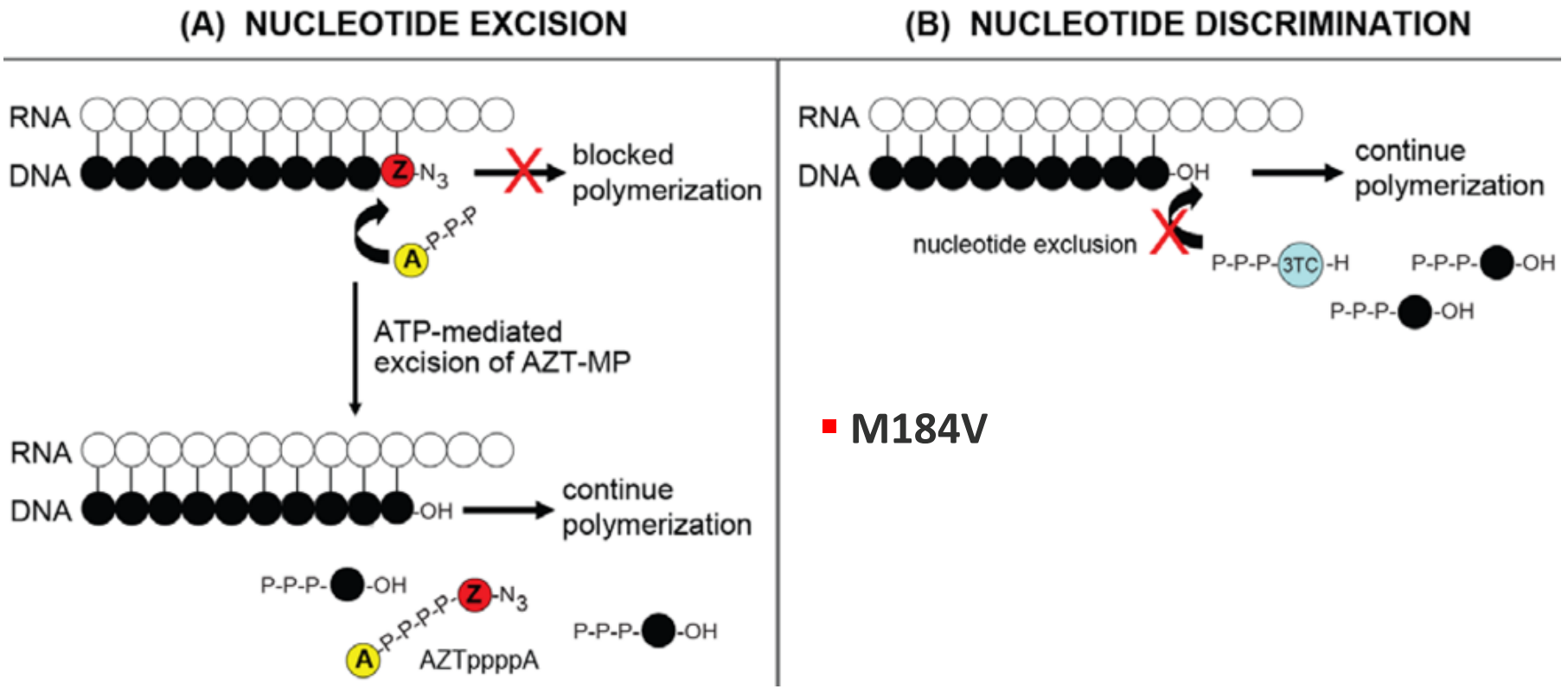
Mechanisms of NRTI resistance



- M184V (3TC, FTC)

- T215Y (AZT, ABC, ddI, d4T, TDF)

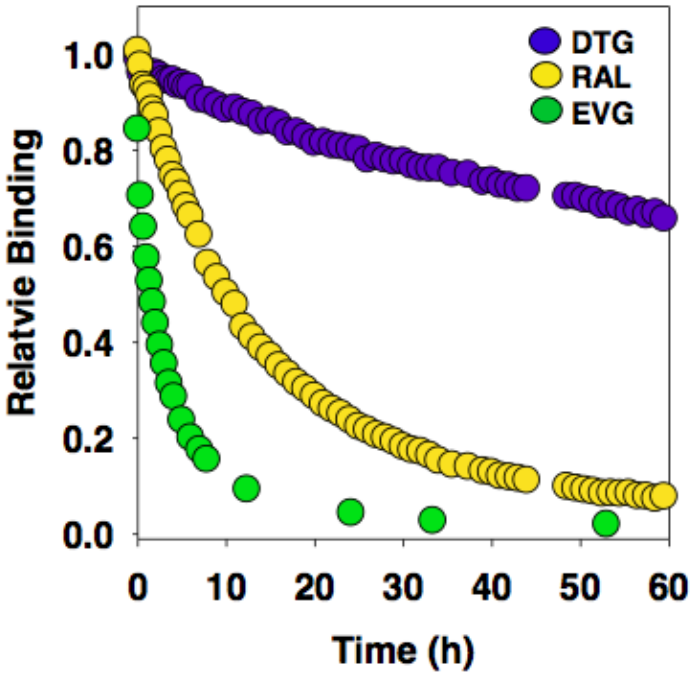
Mechanisms of NRTI resistance



- T215Y
- M184V

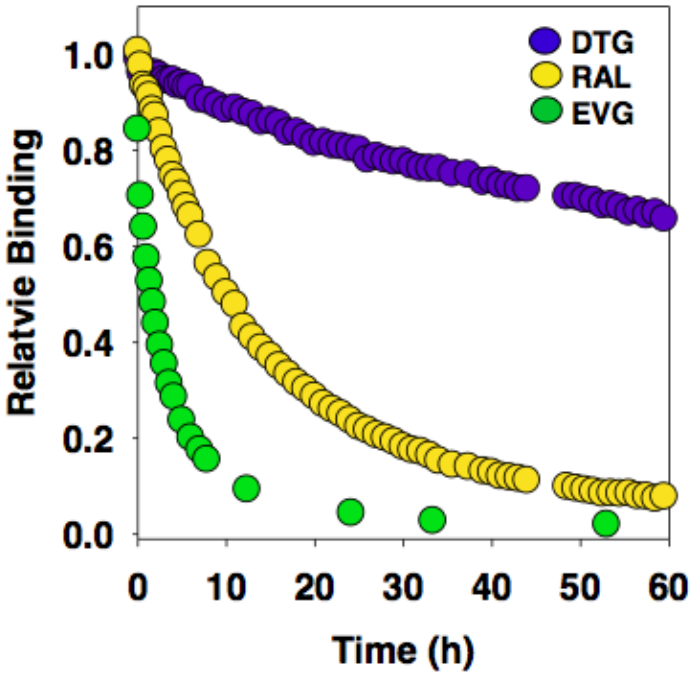
Antagonised by M184V

Dissociation time of integrase inhibitors

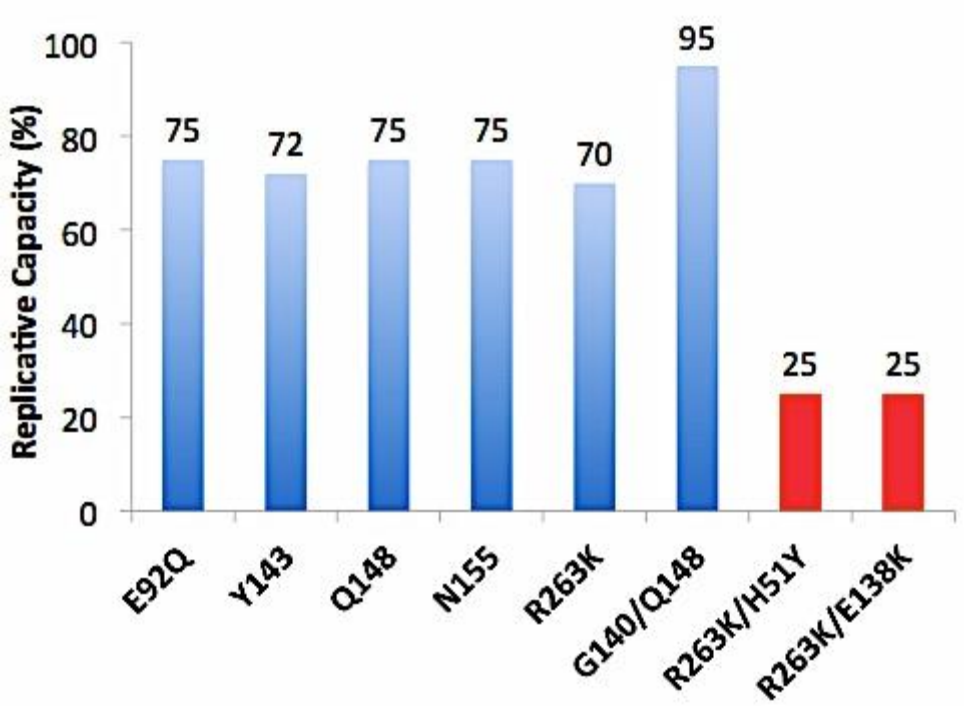


Hightower Antimicrob Agents Chemother 2011; Quashie J Virol 2012; Wainberg ISHEID Conference 2014; Doyle JAC 2015

Dissociation time of integrase inhibitors



Replicative capacity of integrase mutants



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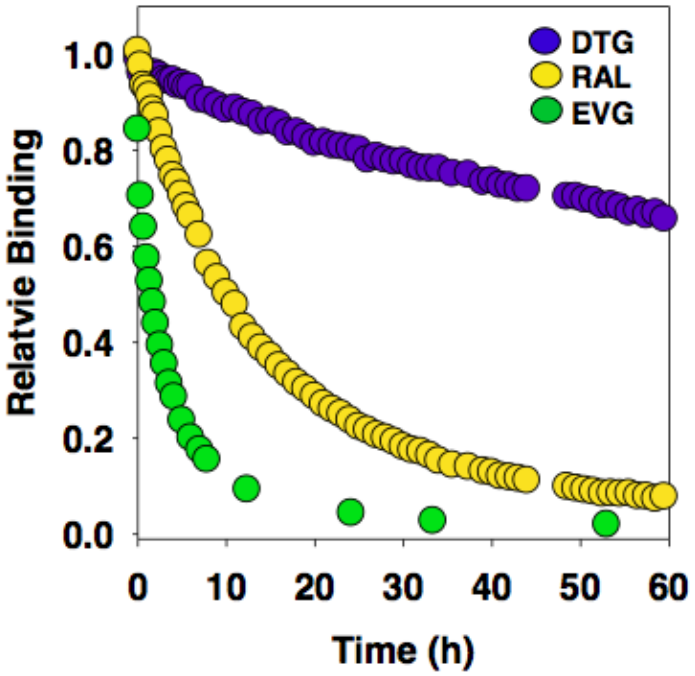
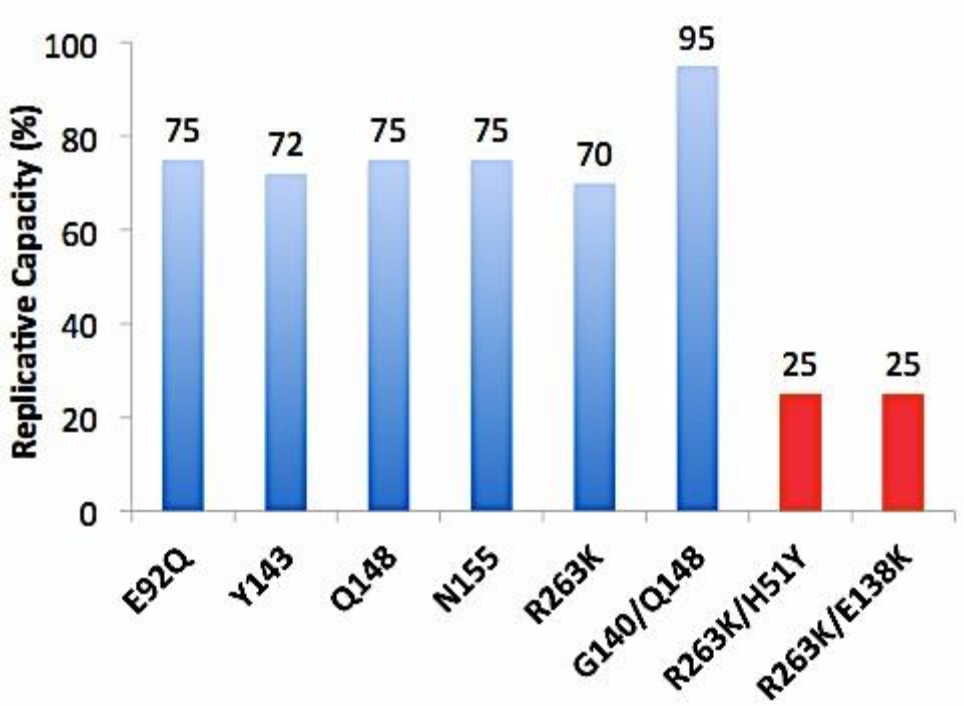


Replicative capacity of integrase mutants



GGT or GGC → Glycine ← GGA or GGG

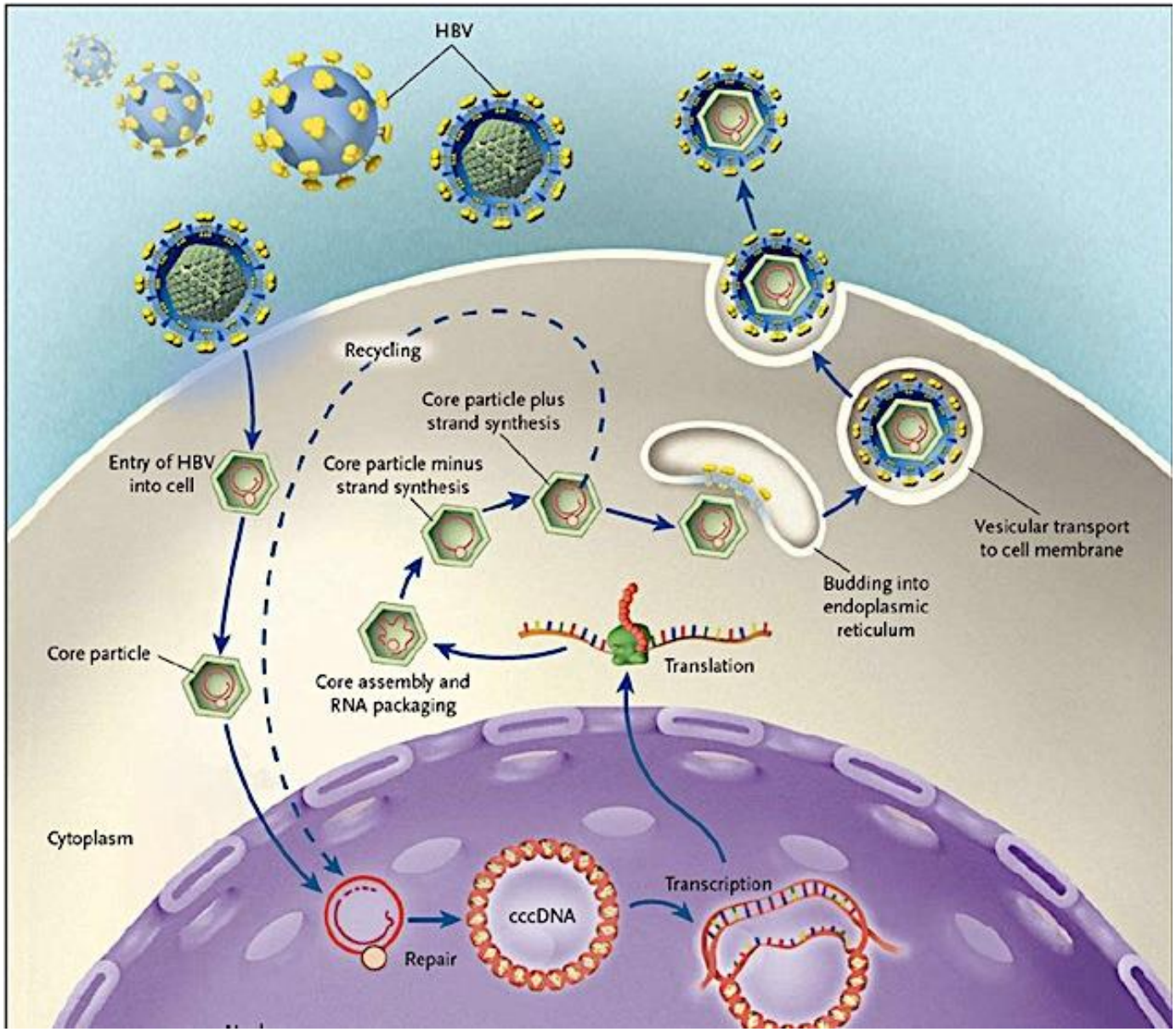
subtype B — AGT or AGC — non-B subtypes

Serine

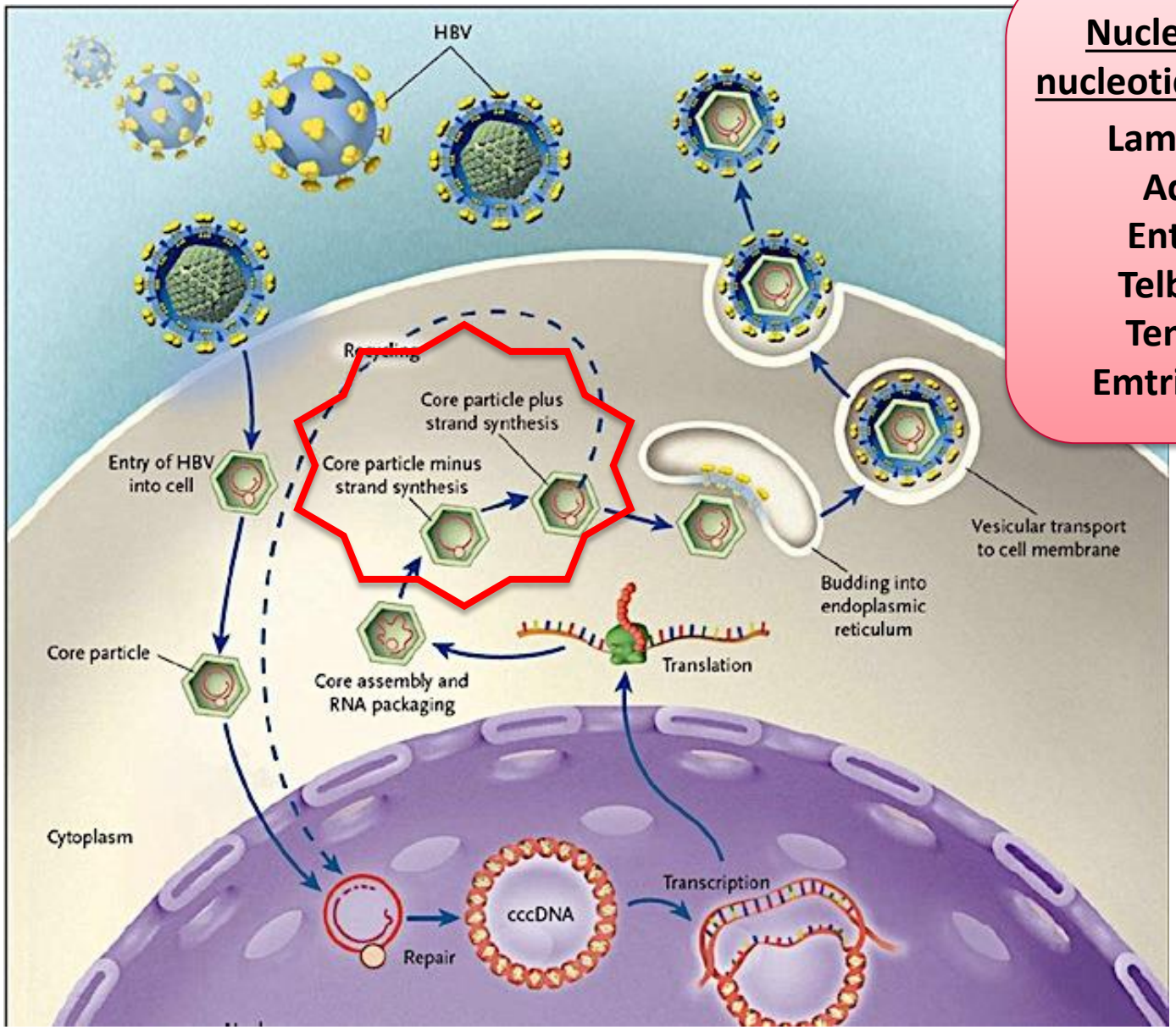
Codon usage at integrase position 140 in B vs. non-B subtypes

Hightower Antimicrob Agents Chemother 2011; Quashie J Virol 2012; Wainberg ISHEID Conference 2014; Doyle JAC 2015

HBV replication



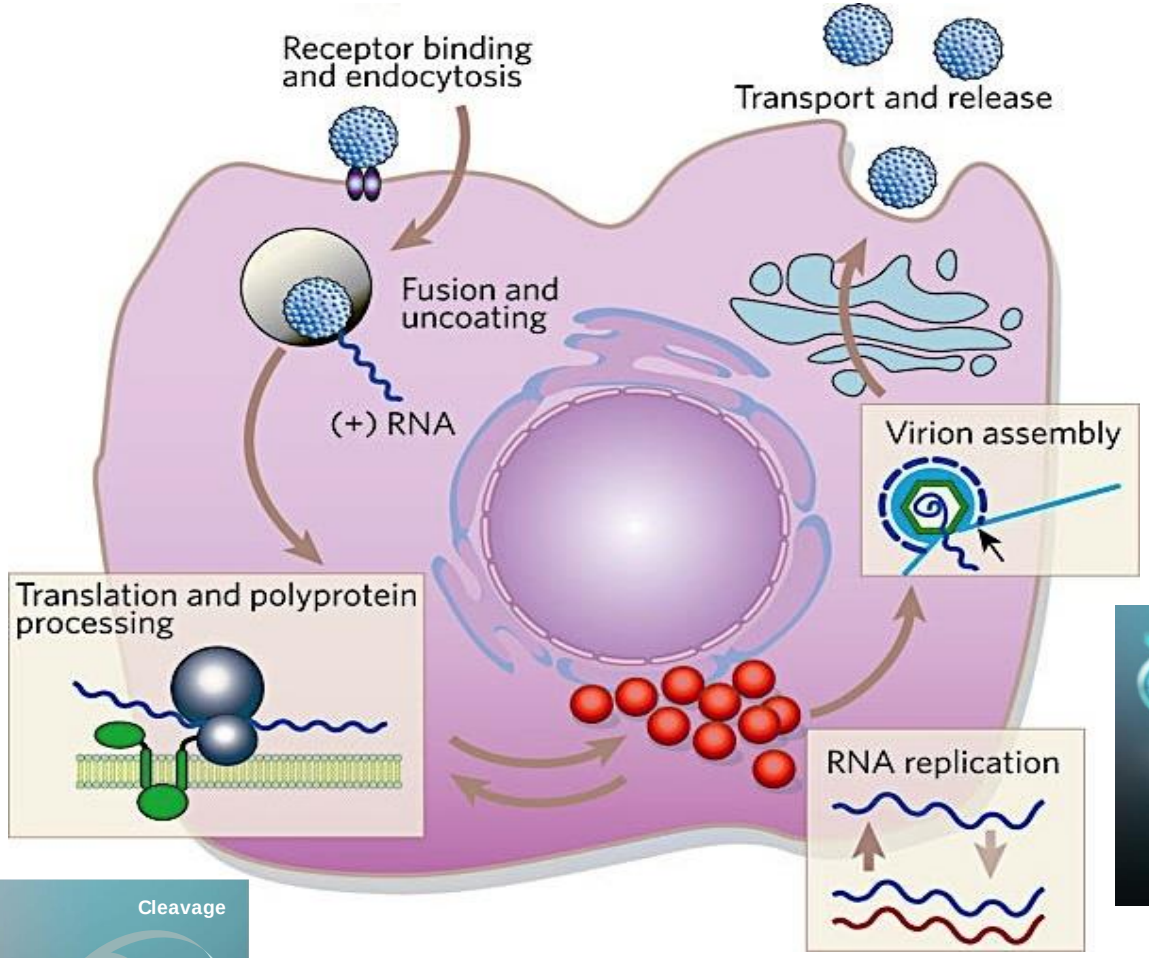
HBV drug targets

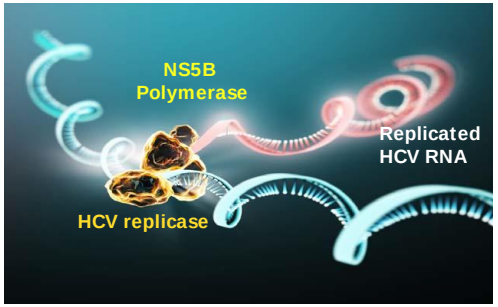


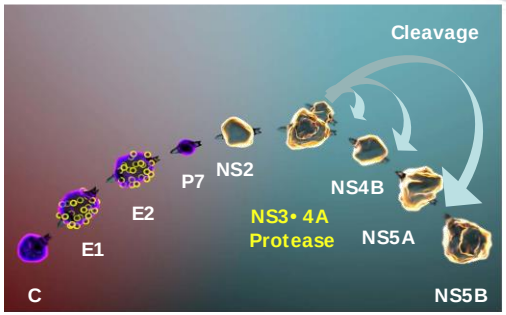
Nucleoside and nucleotide analogues

Lamivudine*
Adefovir
Entecavir*
Telbivudine
Tenofovir*
Emtricitabine*

HCV replication

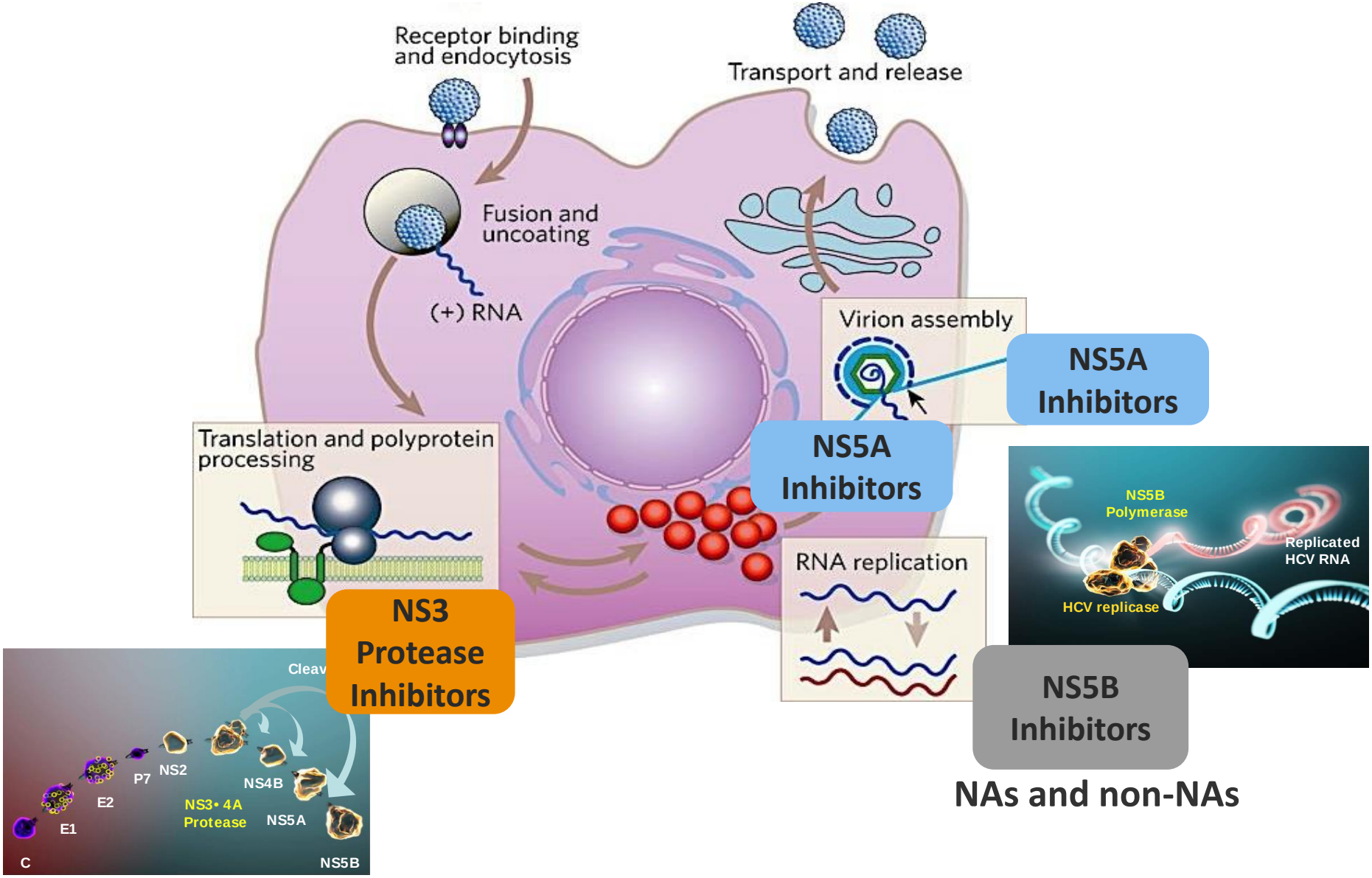




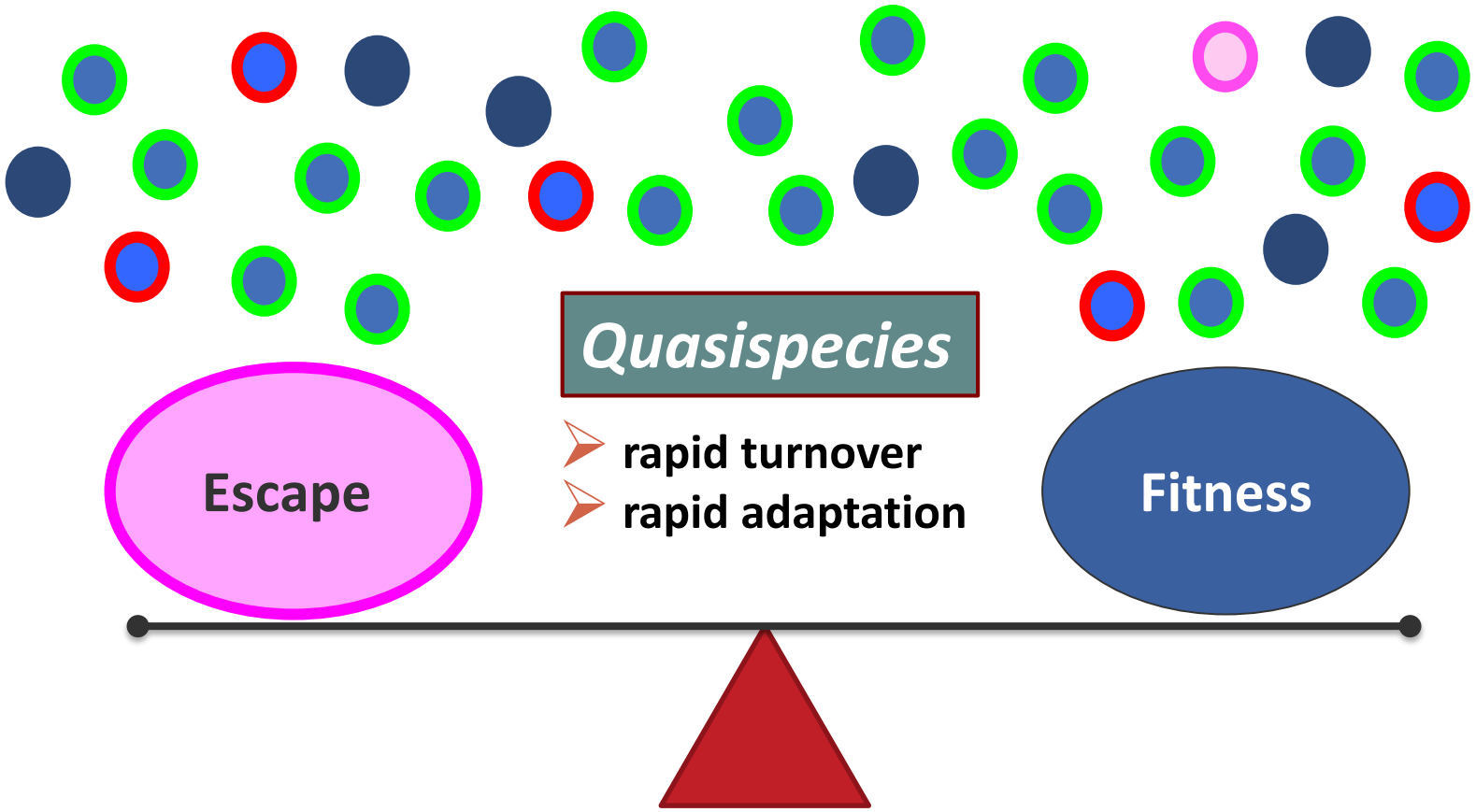


Brett Nature 2005

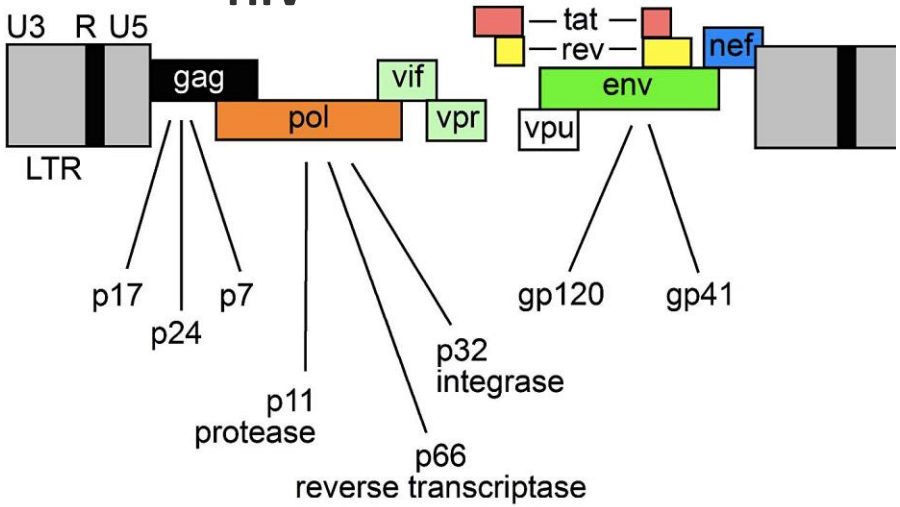
HCV drug targets



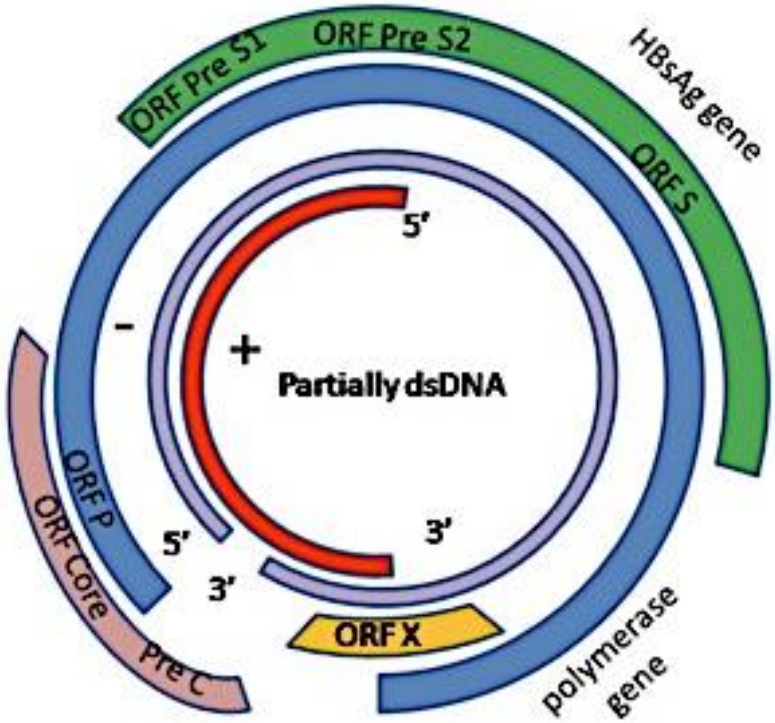
- Rapid replicating virus (HIV 10^{10} - HBV 10^{11} - HCV 10^{12} particles/day)
- Rapid clearance of newly produced virus
- Highly error prone polymerase ➔ High mutation rate
- Some mutations are detrimental, some allow escape



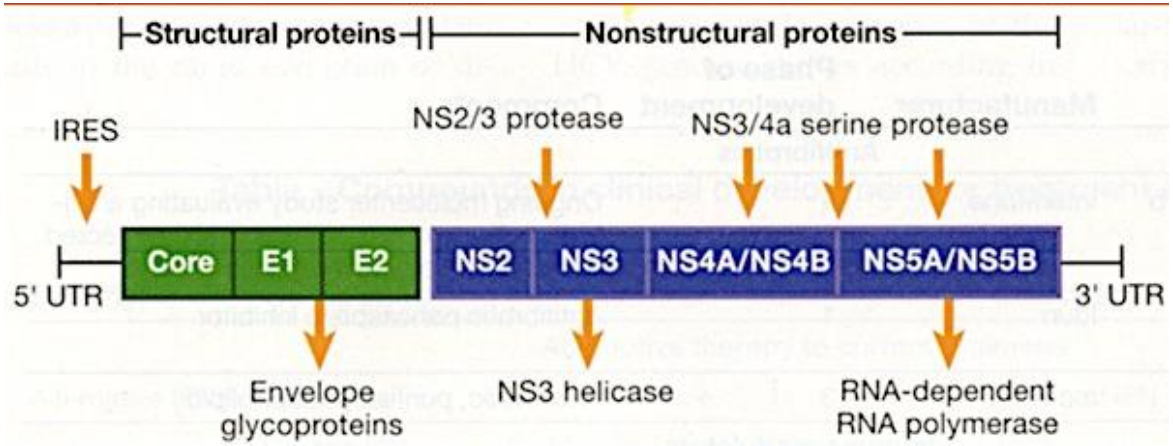
HIV


U3
R
U5
LTR
gag
pol
vif
vpr
tat
rev
nef
env
vpu
p17
p24
p7
p11
protease
p32
integrase
p66
reverse transcriptase
gp120
gp41

HBV

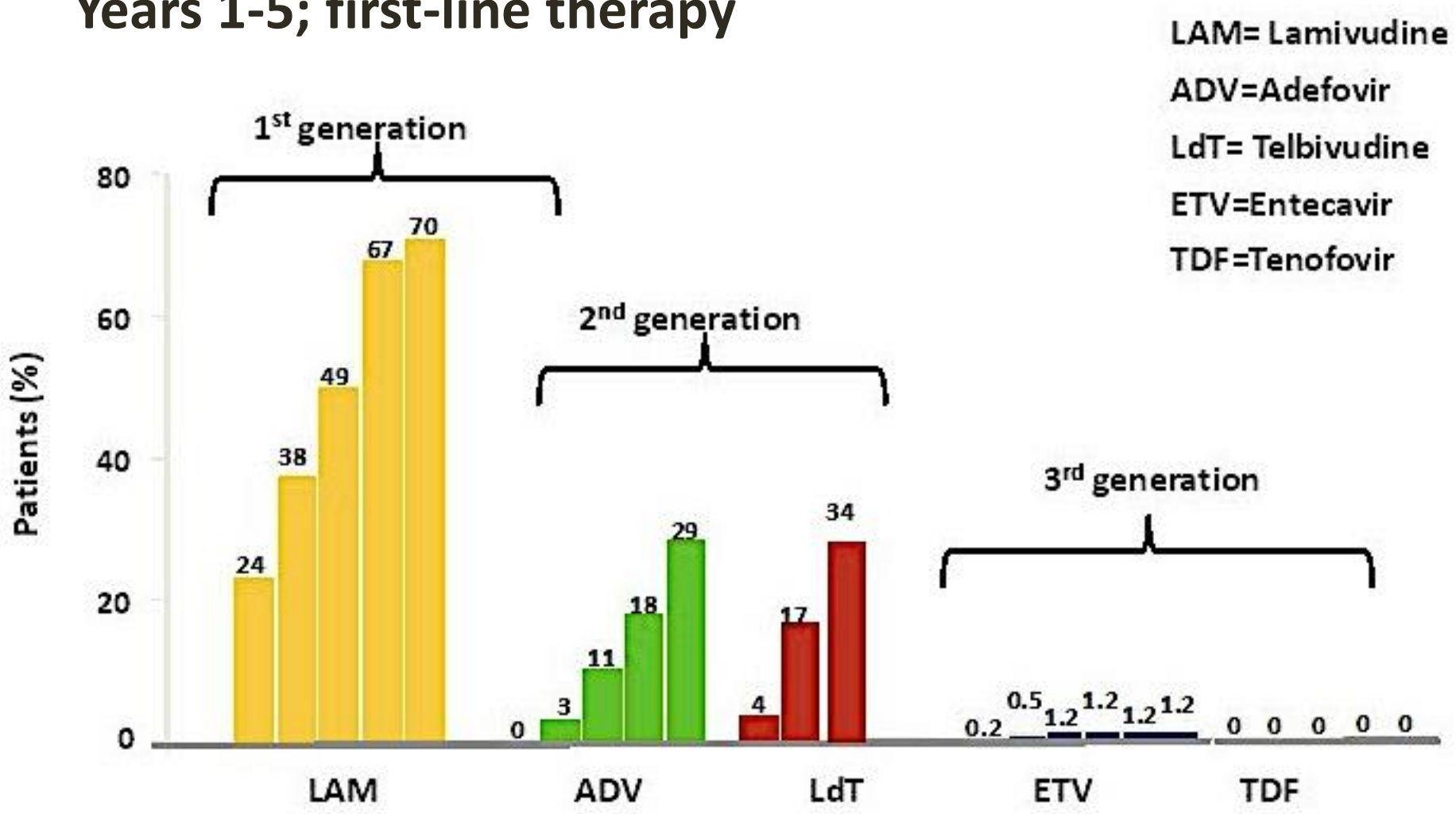

ORF Pre S1
ORF Pre S2
HBsAg gene
ORF S
5'
-
+
Partially dsDNA
ORF P
ORF Core
Pre C
5'
3'
3'
ORF X
polymerase gene

HCV

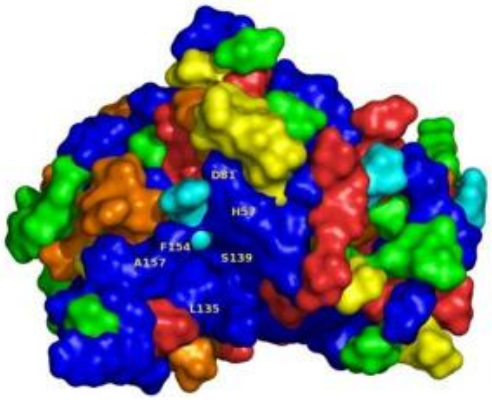

Structural proteins
Nonstructural proteins
IRES
NS2/3 protease
NS3/4a serine protease
5' UTR
Core
E1
E2
NS2
NS3
NS4A/NS4B
NS5A/NS5B
3' UTR
Envelope glycoproteins
NS3 helicase
RNA-dependent RNA polymerase

Incidence of HBV drug resistance

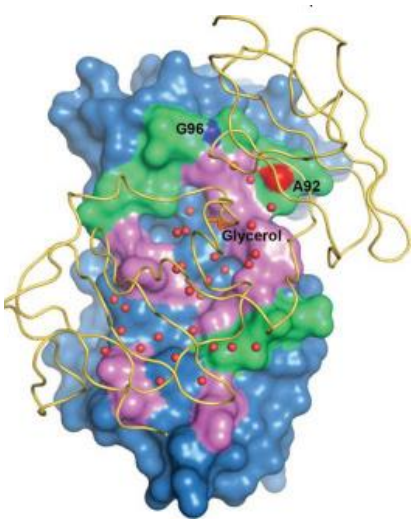
Years 1-5; first-line therapy



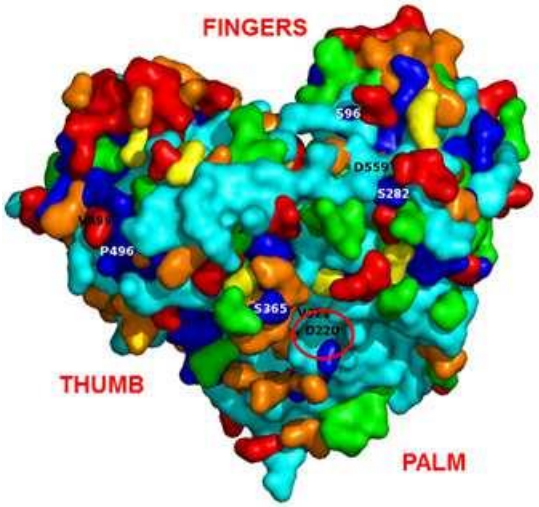
HCV genetic variability



NS3: 42% of amino acid conserved among all genotypes



NS5A: 46% of amino acid conserved among all genotypes



NS5B: 55% of amino acid conserved among all genotypes

Prevalence of NS3 resistance mutations in naïve patients with HCV Gt1a

RAMs	CS & DS >10% n=238	DS 1-10% n=178
	n (%)	n (%)
36M	1 (0.4)	2 (1.1)
36L	5 (2.1)	0 (0)
54S	9 (3.8)	0 (0)
55A	10 (4.2)	0 (0)
80K	44 (18.5)	0 (0)
168E	1 (0.4)	0 (0)
170A	1 (0.4)	0 (0)
170T	0 (0)	2 (1.1)

RAMs = Resistance-associated mutations

- HCV treatment-naïve subjects
- Tested by Conventional Sequencing (CS) and Deep Sequencing (DS, Illumina)
- Most mutations detected by CS (= dominant)
- No difference in HCV RNA load in samples with vs. without resistance (= preserved fitness)

Beloukas Clin Microbiol Infect 2015

Profile of HCV treatment options

	TARGET of THERAPY				
	Protease 1st gen	Protease 2nd gen	NS5A	NS5B NAs	NS5B non-NAs
Resistance profile	■	■	■	■	■
Genotype coverage	■	■	■	■	■
Potency	■	■	■	■	■

■ Least favourable profile
■ Average profile
■ Good profile

Key points:
Drug resistance with HIV, HBV, HCV

- Drug-resistant mutants emerge ”spontaneously “ during virus replication
- HIV and HBV mutants exist as rare species prior to therapy
- HCV single/double mutants are often dominant in naïve patients (NS3 and NS5A)
- Virus replication under drug pressure drives expansion of the mutants – *Natural evolution* ➔ *increasing resistance & fitness*
- If therapy is stopped, drug susceptible virus tends to outgrow resistant mutants selected by therapy – *mutants persist as enriched minority species*
- Mutants are archived in HIV DNA provirus and HBV cccDNA

Your turn ☺

Which of the following correctly describes HIV?

1. **RNA virus, high replication during AIDS phase only**
2. **RNA virus, high replication, stable genetic make-up**
3. **RNA virus, high replication, rapid genetic evolution**

Your turn ☺

Which of the following correctly describes HIV?

1. **RNA virus, high replication during AIDS phase only**
2. **RNA virus, high replication, stable genetic make-up**
3. **RNA virus, high replication, rapid genetic evolution**

Your turn ☺

Which of the following correctly describes HBV?

1. **HBV polymerase lacks reverse transcriptase activity**
2. **The genomic structure favours rapid emergence of resistance**
3. **Resistance is less of a problem with 3rd gen drugs**

Your turn ☺

Which of the following correctly describes HBV?

1. **HBV polymerase lacks reverse transcriptase activity**
2. **The genomic structure favours rapid emergence of resistance**
3. **Resistance is less of a problem with 3rd gen drugs**

Your turn ☺

Which of the following correctly describes HCV?

1. **Resistance is created by suboptimal therapy**
2. **Resistance is selected by suboptimal therapy**
3. **Resistance is archived in the nucleus of hepatocytes**

Your turn ☺

Which of the following correctly describes HCV?

1. **Resistance is created by suboptimal therapy**
2. **Resistance is selected by suboptimal therapy**
3. **Resistance is archived in the nucleus of hepatocytes**


Well
done!

The HIV virology timeline

HIV-1 isolated

HIV-1 genome sequenced

HIV replicates at high levels throughout the infection

HIV replication drives immune compromise

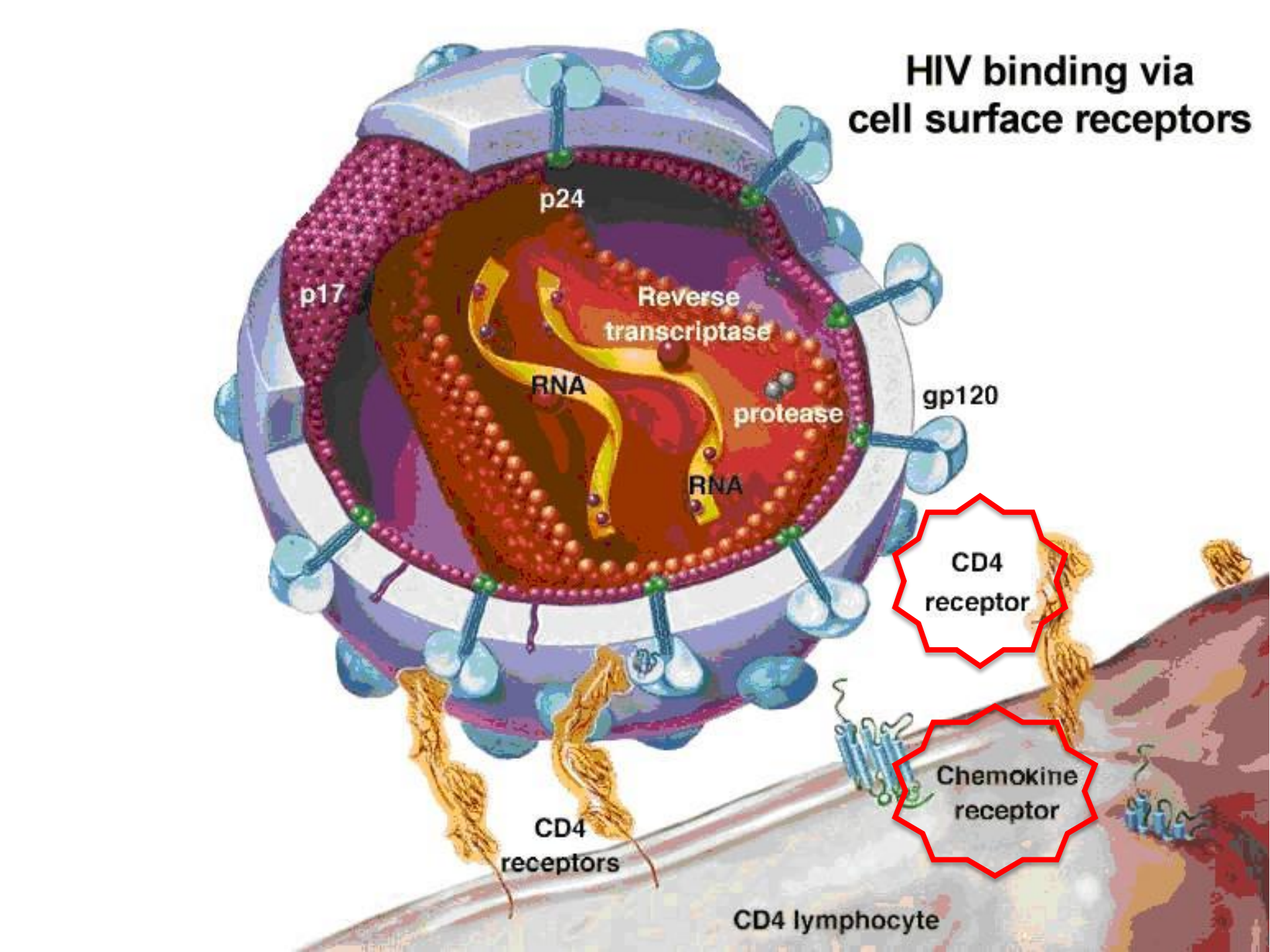
Highly active antiretroviral therapy

Plasma HIV RNA ('viral load') suppression as goal of therapy

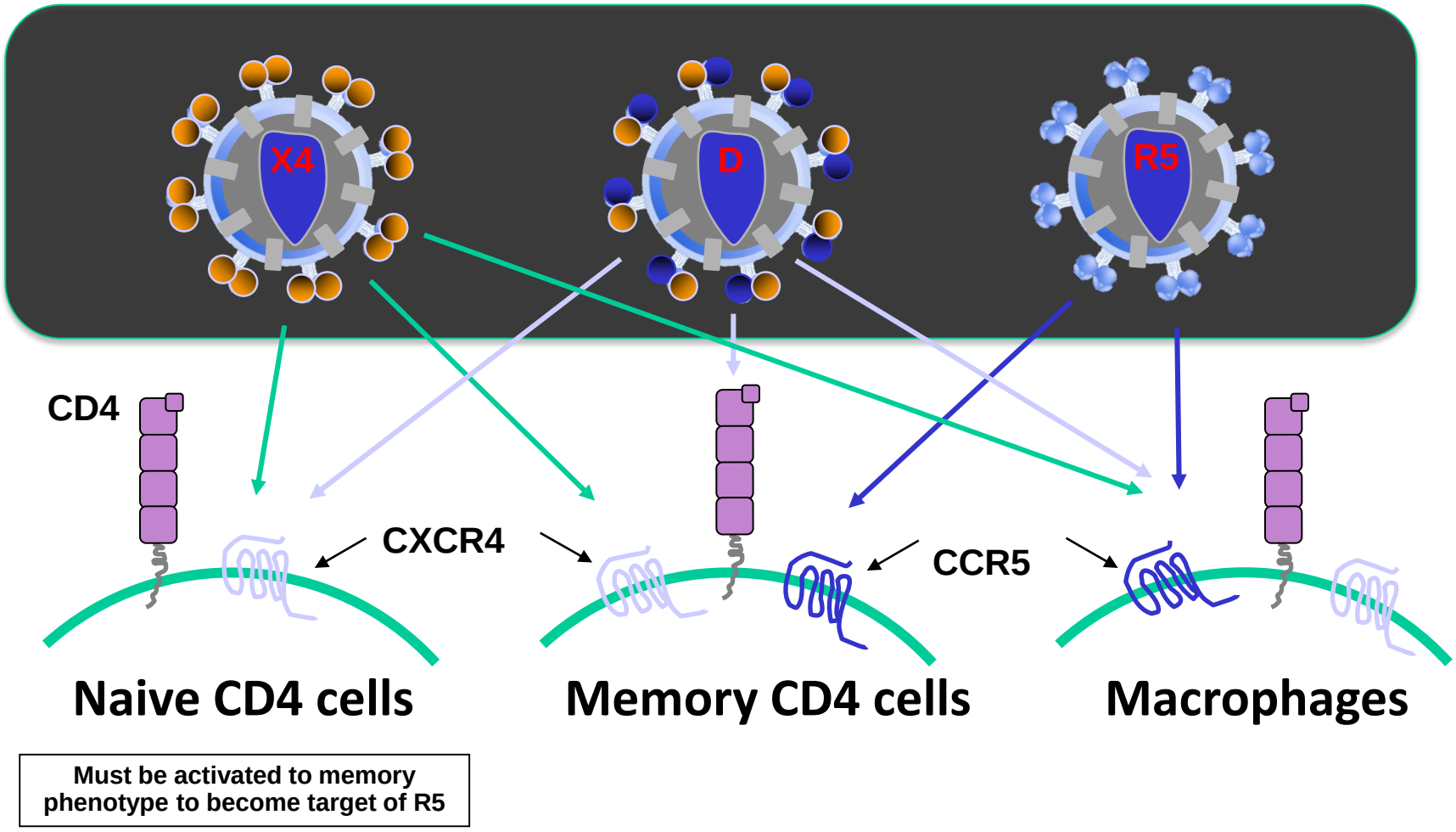
HIV replication causes disease through immune activation & inflammation

HIV eradication research

1982 1985 1991 1995 1996 2009 2010 →


HIV binding via
cell surface receptors
p24
p17
Reverse
transcriptase
RNA
protease
RNA
gp120
CD4
receptor
Chemokine
receptor
CD4
receptors
CD4 lymphocyte

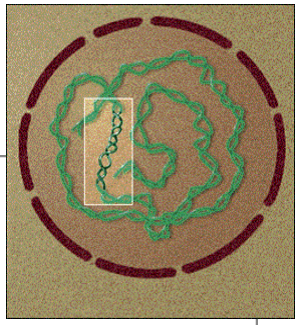
HIV tropism defined by co-receptor use

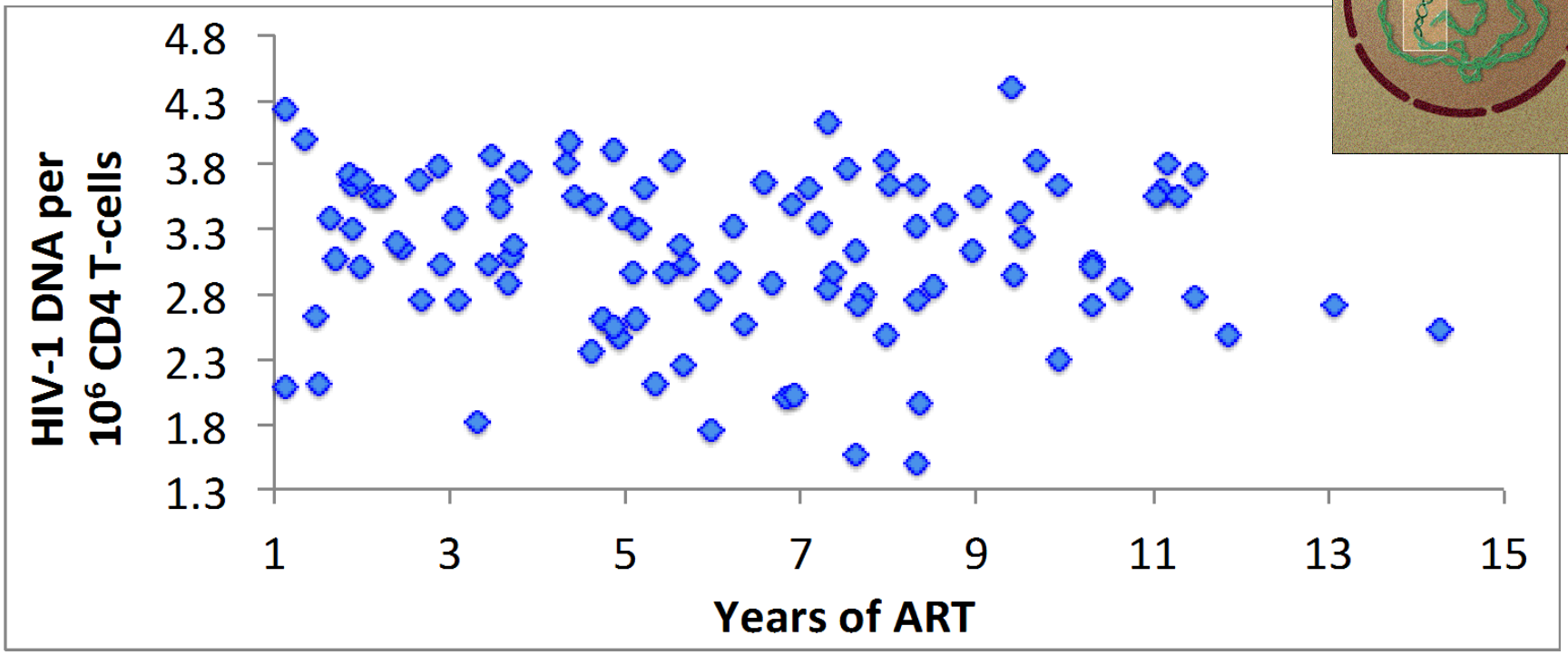


Esté Lancet 2007

HIV DNA load during antiretroviral therapy

- HIV DNA quantified in PBMC

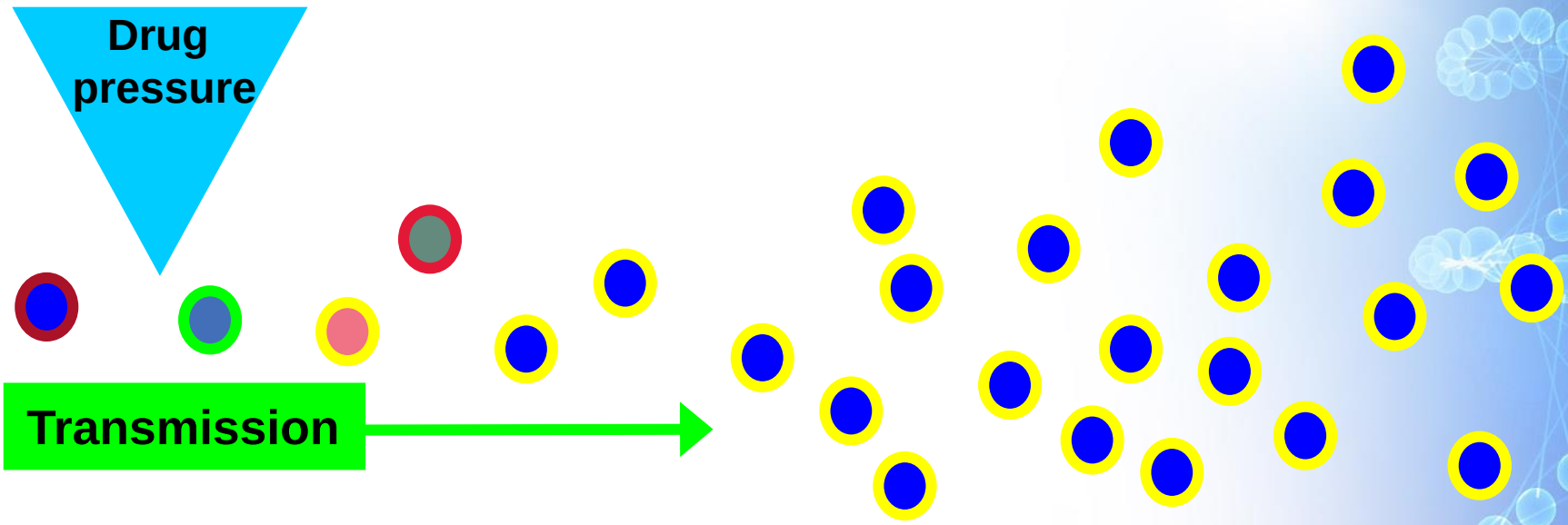




Geretti et al. International Workshop on HIV & Hepatitis Viruses Drug Resistance 2013

Genetic barrier and cross-resistance

Class	ARVs	Genetic Barrier	Cross Resistance
NRTIs	ZDV/3TC, d4T/3TC	+/++	+++
	ABC/3TC, TDF/3TC	+	+++
	TDF/FTC	+/++	+++
NNRTIs	EFV, NVP, RPV	+	+++
	ETR	+/++	++(+)
PIs	Unboosted	+/++	++/+++
	Boosted	+++/++++	+/++
Fusion inhibitors	T20	+	NA
CCR5 antagonists	MVC	+/++	NA
Integrase inhibitors	RAL, EVG	+	+++
	DTG	++/++(+)	++(+)



Transmitted Drug Resistance

Relatively stable after transmission
Gradual reversion over time
Persistence at low frequency in plasma
Persistence in latently infected cells